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STUDIES

FROM THE

OTHO S. A. SPRAGUE MEMORIAL INSTITUTE

COLLECTED REPRINTS

VOLUME V

CHICAGO
1917

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GOLD THERAPY OF TUBERCULOSIS* †

By LYDIA M. DeWITT, M.D.

Chicago

In 1890, Robert Koch published the statement that gold salts and especially the gold cyanides had a high inhibitory power over the growth of the tubercle bacillus in the test tube, a dilution of 1 in 2,000,000 completely preventing the growth of this organism in suitable media. He also stated, however, that these substances have no such influence in the animal body. Pharmacologists generally consider gold less toxic than mercury, silver, and many other metals and state that small amounts may be taken for many months without ill effects. The lethal dose of gold potassium cyanide when given intravenously is given by Bruck and Glück as 15 mg. per kilo of rabbit, the toxic dose being ten mg. per kilo. One milligramme per kilo every second day caused no symptoms, while two mg. per kilo every second day caused emaciation and finally death with fatty liver and kidney. Heubner states that 15 mg. of the salt or seven mg. of gold per kilo of rabbit causes death in a few minutes from respiratory stasis, the blood pressure rapidly falling to zero and all organs showing hyperaemia with occasional ecchymoses and larger hemorrhages. Carnivora stand about three times as much as herbivora. Heubner believes that gold salts exercise a toxic influence on the walls of capillaries and veins. Gold cyanide was recommended for internal use in pulmonary tuberculosis by Chrestien over 60 years ago (Oesterlin's *Hand. der Arzneimittel*).

Bruck and Glück in 1913, finding continued treatment by intravenous or intracardiac injections not feasible in guinea pigs, treated by intravenous infusion 21 tuberculosis patients, mostly lupus, with gold potassium cyanide [$AuK(CN)_2$] alone or combined with tuberculin and obtained good results. Junker and Bettmann report similar results from the use of gold potassium cyanide and Arthur Mayer found gold cyanides either alone or combined with borcholin to have a favorable influence in patients. The borcholin is believed to dissolve the fatty sheath of the tubercle bacillus.

In 1891 Liebreich noted that cantharidin caused a local reaction in tubercles and other inflammatory foci. This was verified by Feldt, who found that by condensing it with ethylenediamine he could reduce its toxicity about 680 times without affecting its affinity for the tubercle. Spiess and Feldt have published several reports on experiments on the use of a gold cyanide salt of this cantharidinethylenediamine. They first

* From the Otho S. A. Sprague Institute and Pathological Department of the University of Chicago.

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used the auric salt but found the aurous salt more effective and have for several years used the cantharidinethylenediamine-aurous cyanide under the trade name aurocantan in the treatment of tuberculous animals and men. This preparation and other gold preparations were first tested on over one hundred tuberculous animals, two mg. per kilo being the dose well borne. A local reaction consisting of hyperaemia and hemorrhage around the nodes followed the injection. Compared with the control animals, the treated animals increased in weight, lived many months longer and showed less extensive disease in the internal organs, while many tubercles were encapsulated by connective tissue. They state that subcutaneous injections were less effective than intravenous because the gold was anchored to the body cells long before it could reach the tubercles and the bacilli. After one and one-half years of experimentation on animals, they began to employ it in the treatment of tuberculous patients, using from 25 to 100 mg. usually with no ill effects. In a report published by Spiess and Feldt in 1914, they stated that they had then treated one hundred tuberculous patients with the aurocantan by intravenous infusions. Symptoms of reaction began some hours after the injection. These were elevation of temperature with sometimes nausea, vomiting and diarrhoea. By analysis they found that after injection of 15 to 20 mg. of gold at intervals of four or five days, gold was excreted through the urine and feces at the rate of one to two mg. daily. Most patients note at once a subjective improvement in their condition, suggesting that a specific detoxication of the specific toxins of the disease was taking place. In some cases a strongly positive von Pirquet reaction diminished and finally became negative under the treatment; in others, the reaction was unchanged. In some cases a combination of aurocantan with tuberculin gave good results and in others the ultra violet rays were used in combination with the aurocantan. They suggested that a combination treatment promises better effects than the simple treatment with one chemical. In lupus and in laryngeal tuberculosis the local effects were most pronounced. In pulmonary tuberculosis the process was distinctly influenced, especially during the reaction time. Increased cough, increase of sputum, changed breath sounds and râles give evidence of a reaction. Of the 100 cases, 80 had laryngeal, generally with pulmonary tuberculosis, five had lupus and two had vertebral caries. Spiess and Feldt are very conservative and careful in their statements regarding permanent cure or even permanent improvement, but state that their gold therapy offers a better guarantee for permanent cure than other local methods of treatment. Ninety of the 100 cases were reported as decidedly improved and many as clinically healed. Spiess and Feldt affirm that the influence of the various gold preparations is largely, if not wholly, based on the oxygen carrying and oxidizing power of the gold, while in their preparation the cantharidin acts as a transporter for the gold. They think there is a direct action on the bacilli, reënforced by anti-bodies, by increased resorptive activity of the cells of the tubercle and by stimulation of connective tissue formation. Recently a Japanese

investigator, Munemasa Tsuzuki, has suggested the use of Aurolin, $AuCl_3(NaCl)_2$, $Au(OH)_3$, $CuCl_2H_2O$, in the treatment of tuberculosis. The fatal dose is 250 mg. to a kilo of guinea pig. Tuberculous guinea pigs were treated by subcutaneous injections and outlived the control pigs. Of 40 patients treated in this way for thirteen months, 18 showed decided improvement. He thinks that the effect is due not so much to its direct action on the tubercle bacillus as to the formation of connective tissue as a result of its local irritating action.

My own work on the gold therapy of tuberculosis has extended over the last two years and has been carried on practically exclusively on tuberculous guinea pigs. I began my experiments with the ordinary gold cyanides $AuK(CN)_2$, $Au(CN)_3$ and AuCN. As the table will show, life was not prolonged and the disease was not checked as compared with the control animals. The average duration of the disease in the untreated controls was considerably longer than in the treated animals, even when we leave out of consideration the considerable number of animals which died early from incidental infections, from hemorrhage or from toxic doses of the gold. The extent of the disease in the two sets of animals was also about the same. The gold was administered by intracardiac, intramuscular and subcutaneous injections at intervals, with feeding of the gold salt on all days when there was no injection. The subcutaneous injections caused considerable necrosis and the intracardiac were by no means without danger; the treatment apparently had no beneficial effect on the progress of the disease.

As it was impossible on account of the war to obtain from Germany any of the aurocantan used by Spiess and Feldt and as I wished to try their preparation, some was made for me by my assistants according to the German patent. This preparation, $N(CH_2)NH_2.HAu(CN)_2$, has shown much less necrotizing action than the other gold salts and could be injected subcutaneously for long periods with no material injury to the skin. At first it was administered only by intracardiac injection but later it was found that after subcutaneous injection the excretion and distribution were the same and about as rapid as after intracardiac injection. On account of the greater safety and convenience therefore subcutaneous injections have been used exclusively in all the later experiments. As it was found that only about one-half of the gold injected was excreted in the course of one week the injections were made once a week, thus keeping up a concentration in the body much greater than should be necessary to prevent the growth of the tubercle bacillus. The treatments were always begun about 24 hours after inoculation. The organism used in the experiments with gold cantharidin were much less virulent than in the others so that many of the controls failed to develop a generalized disease even after 299 days. As is seen from the table, the duration of life is much less and the extent of the disease greater in the treated than in the untreated animals. It may be stated, however, in agreement with the findings of Spiess and Feldt, that there is a tendency to an increase of weight in animals treated with gold canthari-

din, this tendency being in the main slightly more pronounced in tuberculous than in non-tuberculous animals. This increase of weight usually continues until a short time before death.

As it has been frequently stated that it is unfair to judge of the pharmacologic effect of a drug on man by its effect on guinea pigs, I have tried to determine the distribution and fate of the gold in the treated animals, since it is fair to assume that these will be the same in human as in animal tissues. If gold shows no specific attraction for tuberculous tissues in the guinea pig, it is quite unlikely that it will have any for human tubercles.

Since no method of quantitative chemical analysis was known which was delicate enough to determine the small amounts to be found in the tissues of these animals, a method was developed by my assistant, Mr. Cadwell, which will soon be published by him in detail. In brief, however, the method is as follows: The tissue is digested with concentrated sulphuric and nitric acids, the acid evaporated down to about 1 cc. and the residue treated with aqua regia to insure the oxidation of the gold. The free Cl is removed with ammonia, which is neutralized with HCl. It is then filtered through asbestos and treated with ammonia, and HCl again to slight acidity. Phosphoric acid and disodium phosphate are added and the solution made up to 40 cc. and electrolyzed at 60 degrees and with a voltage of 1 to 1.2 volts.

By repeated analyses of gold cantharidin animals, it was determined that an average of 40 to 50 per cent. of the gold was excreted in the urine and feces in one week after injection, the percentage being about the same when the urine and feces were saved for two months and analyzed. The largest amount was usually excreted on the third and fourth days after injection and usually rather more in the urine than in the feces. The amount found in the organs varies from six to 14 per cent. The remainder of the gold, averaging about 26 per cent., was found distributed through the rest of the body. The gold potassium cyanide animals showed a slightly different distribution. They excreted on an average only 17.55 per cent., while 16 per cent. was found in the organs and about 59 per cent. in the rest of the body. Of the organs the spleen usually contained more gold than any other organ in proportion to its weight; the liver, kidneys, lymph glands and the skin around the point of injection came next and contained about the same amount per gram weight of tissue, while the lungs, the intestines and the rest of the body were much alike and contained the least per gram of tissue. This distribution of gold is noteworthy, inasmuch as copper, mercury and other metals, so far as published analyses indicate, have shown a marked predilection for the liver, while the spleen contained very little, if any.

The points of greatest interest in these analyses are: First that there is a very wide distribution of the gold in the body, only the brain containing none, while the blood and heart have practically none, except shortly after intracardiac injection, and secondly that there is no marked difference between the tuberculous and the non-tuberculous animals. In

the cases in which a difference could be noted, the tuberculous organs contained less gold than the normal organs. Certainly it can be said that gold has shown no specific affinity for tuberculous tissue.

Furthermore even the smaller amounts found in liver, spleen and lungs and other sites favored by tubercles give many times the concentration necessary in the test tube to prevent the growth of the tubercle bacillus. Why then do tubercles grow luxuriantly in organs containing such an amount of gold? Several possibilities present themselves as an explanation for this seeming anomaly:

1. The gold may be so combined with the proteins of the cells or be in such an inert colloidal form in the cells as to be powerless to act on the tubercle bacillus. 2. The bacilli may be so protected from its action by their position in the cells or by their membrane that the gold has no power to act upon them. 3. The bacilli may have acquired a tolerance for the gold.

Average	$C_4N_4H_4Au$		$AuK(CN)_2$		$Au(CN)_3$		AuCN	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control
Days Life	130	218.6	101.7	161.5	155.5	161.5	132	161.5
Extent of Disease	60%	35%	95%	95%	95%	95%	95%	95%
Milligrammes of Gold Received	13.34		150.67		210.95		74.36	
Percentage of Gold Excreted	40.82		17.55					
Percentage of Gold in Organs	9.527		16.4		.72		.465	
Percentage of Gold in Body	26.13		59.48					
Milligrammes Found								
in Liver	.447		.69		.98		.265	
in Spleen	.127		.109		.083		.045	
in Lungs	.071		.168		.105		.12	
in Kidneys	.12		.06		.09		.03	
in Lymph Glands	.053				.02		.09	
in Skin	.45		.27					
Intestines	.26		.69					
Blood	.069							
Body	4.08		4.68					
Milligrammes of Gold per Gramme of								
Liver	.0212		.078		.027		.007	
Spleen	.0916		.1645		.0358		.011	
Lungs	.0105		.0305		.009		.0085	
Kidneys	.0263		.0087		.0165		.007	
Lymph Glands	.0246				.005		.027	
Heart	.009							
Blood	.006							
Skin	.027		0247					
Intestines	.0075		013					
Body	.012		.0145					

Whatever the explanation, these facts seem to make it very improbable that any gold preparation yet used can be efficient against the invasion of the tubercle bacillus either in man or in animals. Furthermore the tendency to hemorrhages following the administration of gold and the fact that it frequently hastens the progress of the disease make its use by no means free from danger. For all these reasons, it would seem the part of prudence to abstain from using gold preparations in our tuberculous patients.

REFERENCES

1. Koch, R.: Deutsche med. Wchnschr., 1890, xvi, 1029.
2. Bruck, Carl, and Glück, A.: München. med. Wchnschr., 1913, lx, 57.
3. Heubner: Arch. f. exper. Path. u. Pharmakol, 1907, lvi, 370.
4. Chrestien: Oesterlin's Handb. der Arzeneimittel.
5. Junker: München. med. Wchnschr., 1913, lx, 1376.
6. Bettmann, M.: Ibid., 1913, lx, 798.
7. Mayer, Arthur: Deutsche med. Wchnschr., 1913, xxxix, 1678.
8. Mayer, Arthur: Beitr. z. Klin. d. Tuberk., 1914, xxxii, 211.
9. Liebreich: Therap. Monatsh., 1891, v, 284.
10. Feldt, Adolph: Deutsche med. Wchnschr., 1913, xxxix, 549.
11. Spiess, Gustav, and Feldt, Adolph: Berl. klin. Wchnschr., 1915, lii, 365.
12. Spiess, Gustav, and Feldt, Adolph: Beitr. z. Klin. d. Tuberk., 1914, xxx, 328.
13. Tsuzuki, Munemasa: Tokyo Med. Weekly, 1916.

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PRIMARY SPONTANEOUS SARCOMA IN MICE[1]

EIGHTH COMMUNICATION

MAUD SLYE, HARRIET F. HOLMES AND H. GIDEON WELLS

From the Otho S. A. Sprague Memorial Institute and the Department of Pathology of the University of Chicago

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The literature on tumors in mice does not furnish satisfactory information concerning the frequency of sarcomas among them. Statistics on other animals indicate that with most species, as with man, sarcoma is much less common than carcinoma. Caspar (1), in his review on the tumors of animals, published twenty years ago, makes the statement that sarcomas behave in animals quite the same as the corresponding tumors in man, and are more malignant than carcinomas, with much more extensive metastasis and rapid post-operative recurrence. They are, according to the literature reviewed by him, the most common tumors of dogs and horses, but it is to be considered that in the earlier literature there are many granulomas mistakenly diagnosed as sarcoma. Frohner (2), in 644 operated tumors in dogs, found 44 sarcomas. In rats, sarcomas seem to be especially frequent. McCoy (3) found, in 100,000 rats killed in plague work, 103 tumors, of which 30 were diagnosed as sarcomas and 18 fibromas. Of the sarcomas, 18 were found in the liver, 5 in the subcutaneous tissues, and 5 in the mesentery, and 1 each in the testicle and the pelvis. This predilection of the liver is corroborated by Woolley and Wherry (4) who found in 22 spontaneous tumors in rats, including 7 sarcomas, 3 sarcomas of the liver, which were, as in many of McCoy's cases, associated with parasites (5). In 250,000 ground squirrels McCoy

[1] Presented before the American Association for Cancer Research, May 8, 1916.

(6) found 8 tumors, all in females. Of these tumors, 5 were diagnosed as sarcomas, there being no carcinomas found in these animals.

Wild animals in general would seem, as far as the very small number of recorded cases indicates, to have a relatively high incidence of sarcoma as contrasted with carcinoma. Of 34 tumors found in all sorts of animals in the Philadelphia Zoological Gardens by Fox (7), there were 18 epithelial tumors and 10 sarcomas. Of these 10 sarcomas, 4 were in birds, and, especially since Rous's studies, the relative frequency of connective tissue growths in birds is generally recognized. But even with the birds eliminated, the relative incidence of sarcoma is high in the wild animals. Of the 6 mammalian sarcomas in Fox's collection, 3 were in wolves, 1 in a male wild mouse (*Peromyscus leucopus*), 1 in a Dorcas goat and 1 in a zebra. The mouse sarcoma was spindle-cell, arising from the soft tissues of the right thigh, and metastases are not mentioned.

The most extensive data concerning spontaneous sarcoma in mice are furnished by Haaland (8). In 288 mice with 353 primary tumors (excluding lymphomatous growths, lung adenomas and sebaceous adenomas), there were 6 sarcomas and 339 epithelial growths. These figures do not give any indication of the actual frequency of sarcomas, as it is expressly stated that "These tumors were found in 288 mice at the time of first entering them in the laboratory register; tumors which developed later, or were only found at post-mortem examination, are not included in these figures (except in a few cases of rare tumors in the internal organs)."

Of Haaland's 6 sarcomas, 3 were typical spindle-cell growths, 1 arising in the pectoral muscles and 2 subcutaneously in the middle of the back; no metastases are described. In addition to these 3 there were: (4) a polymorphous-cell sarcoma which developed in the site of an old operation wound (this mouse also having a carcinoma of the ovary); there was a secondary sarcoma growth in the axilla. (5) An osteosarcoma the size of a "large walnut," in the thigh of a male mouse, recurring rapidly after partial removal but without metastases; this

was inoculated into 800 other animals and round-cell sarcomas were obtained in 15. (6) An old female mouse, recovered from operative removal of a mammary carcinoma, developed a large-cell sarcoma of the spinal column, growing along the cord but not producing metastases. Also to be considered in this connection are the following: (7) A case described as carcinoma with sarcomatous formation, which on inoculation into other mice yielded only sarcomas; (8) a melanoma arising in the site of a slit in the ear of a black female mouse, fourteen months old, with possibly a secondary growth in the neck; inoculation unsuccessful in 121 mice.

The first six tumors were inoculated into 1364 mice, 846 of which lived over four weeks, and of these 68 or 8 per cent developed tumors. The tumors derived from the spindle-cell sarcomas were absorbed again after four to six weeks growth, but if transplanted before spontaneous absorption had begun they could be transplanted indefinitely. It will be noted that of the 6 sarcoma mice, 3 had other tumors, and 2 sarcomas arose definitely at the site of old injuries.

Murray (9) previously reported from the same institution, three cases of sarcoma, but does not indicate the number of mice from which the material was drawn, the number of other tumors in the stock, or other statistics that might be used for comparison. His tumors were: (1) A large spindle-cell intra-abdominal growth surrounding the right kidney, which could not be transplanted (sex of mouse not given); (2) Angiosarcoma or angioma in the left inguinal region of an adult male; inoculated into 82 mice without success. (3) A chondro-osteosarcoma 1.5 cm. in diameeter, from the left groin of an adult female, removed by operation, with recurrence in three months infiltrating the peritoneum. This was successfully inoculated. In structure it resembled the mixed tumors common in the mammary gland of dogs. Haaland (10) has also described a chondro-fibrosarcoma arising from the vertebral column of an old mouse, sex not stated, invading the adjacent tissues but causing no metastasis.

Among Jensen's (11) early papers is described a mouse with spontaneous cancer which died and left four young; one of these

developed an enormous intra-abdominal round-cell sarcoma. Further details are not given.

Jobling (12) described 41 tumors occurring in 26 mice, including 2 sarcomas. (1) Adult female, spindle-cell sarcoma arising below the left foreleg, infiltrating the chest wall and projecting into the pleural cavity; no metastasis. This mouse also had bilateral papillary cystadenomas of the ovaries. (2) Similar in location and structure to No. 1 even to the ovarian adenoma, with a primary lung tumor in addition. Forty mice were inoculated with fourteen takes, "now growing in the second generation."

Tyzzer (13) found one typical sarcoma among 83 primary spontaneous tumors in mice. "This tumor, like several of those already described, occurred in a family derived from a tumor parent." It was in a female, twenty-five months old, was located in the orbit and reached a size of 3 cm. in diameter, penetrating the skull and growing into a vein. From this tumor 11 mice were inoculated, but apparently the tumor was infected and all grafts sloughed out but one, which developed a transitory nodule. The primary growth was composed of atypical cells, mostly larger than connective tissue cells, many attaining the proportions of giant cells, and multinucleated cells were present. Fibroglia fibrils were also described. The lung showed minute metastatic growths. He also described a mouse with many small nodules in the liver, and similar tissue in the spleen, resembling fibrosarcoma, also three other cases with peculiar cellular growths in the liver and spleen that were not classified.

Apolant (14) described a sarcoma arising from the femur in a male mouse, this being the only male with spontaneous tumor in a series of 800 cases. The growth was an osteoid sarcoma arising in the periosteum of the left femur, exhibiting varying structure in different parts, some areas being composed chiefly of spindle cells, while others resembled carcinoma sarcomatodes. Inoculated into 100 mice six takes were obtained, with 100 per cent positive results in later generations, the growth becoming more and more like a round-cell sarcoma.

In the limited material collected above from the literature,

which does not include the cases of sarcoma arising from transplantation of supposedly pure carcinoma, we note the following facts: (1) in proportion to the cases of carcinoma found in mice, sarcoma is very infrequent—much more so than seems to be the case in other animals. Of course the readiness with which mammary gland cancer is recognized elevates that type of tumor into an improper prominence, for our own figures show that when systematic autopsies are performed on mice dying a natural death, other tumors are more frequent than current statistics indicate. (2) As the diagnosis of sarcoma is always the least certain of any differentiation in tumor pathology, and especially so when the numerous peculiar types of granulomatous growths of lower animals are taken into consideration, it is apparent that proper conservatism has been used in animal cancer work, or the proportion of growths called sarcoma would be higher. (3) Of the 17 cases collected, in 2, the sarcoma was definitely recognized as arising at the site of an old trauma, and in 5, other tumors were described in the sarcoma mouse. (4) Sarcomas of mice can be transplanted successfully into other mice, but apparently not more readily than carcinomas, indicating the improbability of infective granulomas having been mistaken for sarcomas. (5) Even in so small a series of cases, an extensive variety of histological structure is seen, spindle-cell sarcomas predominating. (6) Some of the sarcoma mice were males, although as the sex was not stated in all the cases reported, the proportions cannot be determined. (7) In only two of these cases were metastases recorded, indicating that in sarcomas as well as in carcinomas of mice, metastasis is not so frequent as in human cancer. (8) In a few cases the sarcomas were recorded as occurring in "cancer families."

INCIDENCE OF SARCOMA IN THE SLYE STOCK

In 12,000 mice dying in this laboratory, and subjected to careful autopsy, we have found 87 with neoplasms that can be diagnosed as nothing except sarcoma, an incidence of about 7.35 per thousand, including animals of all ages and dying from whatever

cause, whether accidental or natural. We recognize fully the difficulties that attend the differentiation of sarcoma, and for the purpose of this study have excluded every form of new growth concerning the nature of which there seemed any possible room for question. Therefore, we have not included numerous cases in which we think that the growths are probably sarcomatous, and many more in which we cannot be sure that the neoplasm is not sarcoma. The many lymphomatous neoplasms are also omitted, under which term we include not only those conditions which resemble human leukemia and pseudoleukemia, but also many other growths of lymphoid character with marked local infiltrative character but lacking the tendency to generalization exhibited by the foregoing; this latter type of growth corresponds very closely, often exactly, to what are designated as lymphosarcomas in man, and have been observed by all workers with mouse tumors. We also have omitted a series of neoplasms arising in the testicle, ovary and adrenal, which, as with tumors arising from the same tissues in man, often resemble sarcomas closely; for these we prefer Adami's designation of "mesothelioma" and plan to consider them more extensively in a later paper, for they constitute an interesting group.

Having thus omitted every debatable form of neoplasm, and having also excluded every growth that gave any indication of a possible inflammatory origin, we feel assured that these 87 neoplasms are all true sarcomas as judged by the most rigid standards. We may add that numerous borderline cases have been kindly studied by Dr. E. R. Le Count, and in each instance when any one of the examiners was in any way uncertain as to the sarcomatous nature of the neoplasm it has not been included in this series.

On the other hand, the statistical value of our figures is lessened by the fact that we have undoubtedly omitted some growths that are true sarcomas. Our figures represent minimal values only, and especially so as to lymphosarcomas which are excluded entirely. A special study of the lymphomatous and leukemic conditions of mice is now under way, and it is hoped

that when this is completed we shall be able to speak with more assurance as to the identity of growths of this class.

From the standpoint of investigations in heredity, with which our work is particularly concerned, it is just as undesirable to call a sarcoma something else as to include a granuloma among the sarcomas, and hence the rigid classification adopted in this study of sarcomas is no more satisfactory for our heredity statistics than would be a lax classification that included some growths of doubtful nature. Therefore, in charting the heredity statistics it is necessary to recognize the absence of positive criteria for the differentiation of sarcoma, and to admit the borderline cases with a mark of interrogation to indicate this fallibility.

We would also emphasize the character of the material from which these sarcomas have been obtained, and the conditions under which the growths have developed. The 12,000 mice are all the descendants of a limited and carefully selected stock, bred together according to definite plans designed to give evidence as to the influence of heredity upon the incidence of spontaneous tumors in mice, and, hence, including strains of highly cancerous ancestry and strains with ancestry free from cancer. They represent strains in which cancer is very common, strains in which it practically never occurs, and strains of intermediate character. The influence of heredity on the incidence of sarcoma will be considered elsewhere, and we mention these facts here to indicate the character of the material in this respect. It must also be emphasized that none of these mice has been subjected to any artificial influences that might modify its life. In no case is a spontaneous tumor used for inoculation, or operated upon, and no mouse born in this laboratory is ever used for any experimental work whatever. From the moment of its birth every effort is directed to the one object of permitting each mouse to reach a maximum age. Long experience and great care have made it possible to limit to a large extent the epidemic infections that constantly threaten such large colonies of mice under even the best of conditions. Of especial importance is the fact that every mouse that dies is submitted to a

careful post-mortem examination, no matter whether it dies in infancy, from an accident, or from any other obvious cause; and every suspicious area is submitted to microscopic examination by three people or more. Were it not that every dead mouse is thus thoroughly investigated, and that the average age at death is, for a mouse community, very high, we should not have nearly so much tumor material to describe.

Of the 87 sarcoma cases, 30 were males and 57 females, a ratio of almost exactly 1 to 2. These figures may be compared with the statistics of the other tumors of the Slye stock that we have studied in detail; namely, the tumors of the lung and the liver. In the lung tumors (160 cases) there were 42.6 per cent of males and 57.4 per cent of females; in the liver tumors (28 cases) there were equal numbers of each sex. It is evident that these figures fail to bear out the prevalent statements concerning the great infrequency of tumors in male mice. The explanation of the discrepancy undoubtedly lies in the fact that most of the figures in the literature are derived from material selected by external examination, and from breeding stocks with a preponderance of females, so that the common mammary gland tumors constitute by far the greater part of the published material. Our material, coming from mice living out their complete span of life in as favorable conditions as possible, with more nearly normal proportion of sexes, and every death followed by thorough post-mortem examination, gives an accurate picture of the natural proportions of malignancy in the two sexes.

The reason that the females outnumber the males so much much more in the sarcoma series than in either the liver or the lung tumors, is probably that a large proportion of the sarcomas arise in the mammary gland. As we have not yet worked over the entire tumor material from these 12,000 autopsies we cannot state the exact proportion of sarcoma to carcinoma in the mammary gland.

DISTRIBUTION OF SARCOMA

By far the commonest point of origin of these tumors is beneath the skin, including both those that develop from the

subcutaneous tissue proper and those that arise from the mammary gland. Forty-five, or almost exactly one-half, had this origin. Because of the widespread distribution of the mammary gland, it is usually impossible to tell with certainty whether a subcutaneous tumor on the trunk of a mouse arises from the stroma of the mammary gland, or arises from the subcutaneous tissue or muscle fascia and infiltrates the gland tissue. There are certain tumors that arise and develop in such a manner as to indicate strongly that their point of origin is in the mammary gland. When first observed, having then usually the size of a green pea, their location is the same as the carcinomas of the mammary gland, for which they are easily mistaken; like the carcinomas they appear especially in the anterior and posterior pairs of mammae, and a large percentage of those in mated females become apparent at the time of the birth of a litter. With some of them the histological evidence also supports the view that they arise in the mammary gland, because they are largely limited to the mammary gland substance. Often the relation to the tubules is close, resembling in a few instances a pericanalicular fibrosarcoma (fig. 1).

Taking into account the clinical history, the autopsy findings, and the histological evidence of these 45 superficial tumors, 13 seemed certainly to arise in the mammary gland, 10 others probably had the same origin, while with the remaining 22 there was either a lack of satisfactory evidence for this origin, or, in some cases, convincing evidence that the mammary gland was not concerned.

No other one location yielded many sarcomas, but next in order was the osseus and periosteal tissue, with eleven, distributed as follows: Jaw, 1; femur, 4; ribs, 3; foreleg, 2; knee, 1. Several other tumors were attached to bone, but apparently had arisen elsewhere, although in two or three cases this could not be positively determined, as, for example, tumors arising in the orbit.

Other locations which seemed to be the starting point of the sarcomas, as well as could be determined, were: Mesentery, 3;

head, 1; testicle, 1;[2] tail, 1; retroperitoneal, 2; neck, 1; pelvis, 1; ovary, 1; omentum, 1; orbit, 3; abdominal wall, 2; mouth, 1; arm, 1; thigh, 2; scrotum, 2; kidney, 2; uterus, 2; liver, 2; chest wall, 1.

In a few of these instances the tumor had involved several structures so extensively that the point of origin could not be stated exactly, and is only approximated; e.g., neck, pelvis, head, thigh, arm, orbit, chest wall.

UTERINE SARCOMA

The relative infrequency of uterine tumors in all mammals except man is, together with the similar peculiar frequency of human gastric carcinoma, one of the striking features revealed by comparative cancer studies. We have never found a carcinoma that arose primarily in the uterus, nor a leiomyomatous tumor, although Haaland (15) described a typical uterine fibromyoma. In this series, there are, however, two cases of sarcoma of the uterus.

10042. An old female mouse, with senile atrophy of most of the organs, exhibited a bilobed pelvic mass, occupying the site of the uterus. The right lobe measures 20 by 14 by 12 mm., and is pink and of fleshy consistency; the left lobe is paler in color, and measures 18 by 14 by 12 mm. The cut surface yields no exudate. The mass binds together the neck of the uterus, the urinary bladder, and the ureters, but does not involve the vulva (fig. 2). There are no metastases, and the ovaries are not involved. Microscopically all parts of the growth are alike, consisting of a typical spindle-cell sarcoma, with cells densely packed together and but little fibrillar material. In some places the tumor infiltrates the uterine muscular coat and involves the serosa; in others it compresses the muscular wall, as if growing out from the submucosa. Mitotic figures are not numerous.

10828. The right horn of the uterus is enlarged to form a soft fleshy mass, 12 by 10 by 10 mm., which contains no pus or

[2] This was a true spindle-cell sarcoma arising at the site of a wound, and entirely different from the more common "mesothelioma" of the testicle.

products of conception. The left side of the uterus is not involved, and both ovaries seem to be normal. On the diaphragm is a nodule, 2 mm. in diameter, of tissue similar to the horn of the uterus. The regional lymph glands are somewhat enlarged. Death apparently resulted from a suppurative pyelonephritis.

FIG. 2. Sarcoma of the uterus, involving both horns. Part of left horn has been removed for microscopy. Structure large spindle-cell sarcoma.

Microscopically the growth is composed of large spindle cells infiltrating the wall of the uterus in all parts; where under pressure the cells are somewhat smaller. Mitoses are scanty. The diaphragmatic nodule is composed of typical large spindle-cell sarcoma tissue, infiltrating the muscle freely, and with many mitotic figures. The regional lymph glands are hyperemic and hyperplastic, but show no tumor tissue.

It is an interesting fact that in 12,000 autopsies with approximately 2000 tumors there have been but two uterine tumors, both sarcomas, and that these two animals were closely related, 10,042 being the half aunt of 11,828 through the male line. The strain from which they come carries a very high incidence of cancer.

MULTIPLE PRIMARY SARCOMA

There were a few instances in which there seemed to be more than one primary sarcoma in the same mouse. These were as follows:

3117. A male mouse bitten on the back and on the genitals. From each of these sites arose a large spindle-cell sarcoma, alike in structure in each case, but developing synchronously so that there seemed little probability of either being secondary to the other. The genital tumor seemed to arise from the testicle, and was a typical spindle-cell sarcoma, apparently not of the more usual mesothelial type of testicle tumors. There were no metastases.

3444. Female. Here a spindle-cell sarcoma arose on each side of the chest, one being of a larger type of cell than the other. There was a pleural metastasis from the larger cell growth. The difference in structure and clinical course indicated, but did not positively prove them to be independent primary growths.

6629. A female mouse had a large tumor develop on each side of the face. As they arose independently it was thought during the life of the animal that the growths were both primary. At autopsy there was not sufficient difference in histology of the growths to establish this.

9741. Also a doubtful case. A female mouse showed a primary nodule in the ribs, and very soon thereafter a growth in the knee which grew more slowly. Both were osteosarcomas, so it is impossible to be sure whether the knee growth was secondary to or independent of the former.

8289 A female mouse had two perfectly distinct tumors, one arising near the spinal column and extending towards the left axilla, the other arising in the right chest wall. As these are

both spindle-cell sarcomas of similar structure their true independence is not established, although probable.

It will be noted that in all these cases the structure of both tumors in each mouse was nearly or quite identical, so that only gross anatomical and clinical reasons exist for considering them as primary multiple tumors. In the first case the evidence of independence is better than in any of the others. In compiling our statistics each of these cases has been counted as one only, and not as two instances of sarcoma.

CO-EXISTENCE OF OTHER TUMORS

In several instances the mice with sarcomas also had elsewhere in the body a tumor of some other type. This tendency to multiple tumor formation has been noted by all investigators of spontaneous tumors in mice. There were in all 20 such cases, or about 23 per cent of the sarcoma mice. In our series of 160 lung tumors, we noted 23 primary tumors in other organs, or 14 per cent; in the liver tumor series, in 28 mice 7 had tumors in other organs, or 25 per cent. Apparently, then, sarcomas show about the same frequency of co-existence with other tumors as do the hepatic and lung tumors.

Of the 18 co-existing tumors, the lung tumors are decidedly the most common, there having been 11, 5 of malignant character and 6 benign. (The characteristics of these lung tumors are discussed in the fourth paper of this series.) There were 4 mammary gland carcinomas and one tumor of the mammary gland that seemed to be a true benign adenoma. The other tumors were liver adenoma, a squamous-cell carcinoma of the mouth, a perivascular "mesothelioma" of the testicle, and an ovarian adenoma. Not included are two cases of leukemia; there were no cases of pseudoleukemia accompanying typical sarcoma.

In two cases we have what seem to be co-existent sarcoma and carcinoma arising in the same or adjacent tissue, and constituting independent double tumors rather than mixed tumors or carcinoma sarcomatodes.

8560. Spindle cell sarcoma of jaw with squamous-cell carcinoma arising in the overlying mucous membrane. This was an old female mouse of a strain (146) characterized by high cancer incidence. There arose a lump in the lower jaw, the surface of which eventually became ulcerated, but the duration of the tumor was much longer than is usually the case in squamous-cell carcinoma. Microscopically the growth consists of two definite structures (fig. 3). The greater part is composed of a growth resembling typical spindle-cell sarcoma, with many large atypical multinuclear and uninuclear giant cells. Apparently this part of the tumor arose in the periosteum of the mandible, and shows more of the giant-cell sarcoma character than any of the other tumors that we have observed. It infiltrates the muscle and erodes the bones freely. Infiltrating this spindle cell growth from the surface is a typical squamous-cell carcinoma with marked hornification; it seems to have arisen from the mouth epithelium and not the skin. This epithelial growth in places extends quite deeply into the sarcoma-like tissue, and it too erodes and infiltrates bone tissue. There seems to be a slight inflammatory reaction about the epithelial neoplasm within the spindle-cell tissue. Mitotic figures are common in the carcinomatous tissue and much less abundant in the spindle cells. There were no metastatic growths found, but in the lung was a small primary adenomatous nodule. There is no sharp line of demarcation between the two types of growth, and in places there are features suggesting a transition of one into the other. Dr. James Ewing, who has kindly examined this tumor, believes it to be all carcinomatous, and a dentigerous origin is certainly possible. The resemblance to sarcoma is, however, too striking to be entirely dismissed. This growth is not included among our sarcomas, because of the doubt as to its origin.

8889. Spindle-cell sarcoma of the mammary gland adjacent to a carcinomatous growth. A female mouse, with a tumor arising in the left axilla, extending down the entire left arm to the hand, and across the chest to the right axilla. The entire tumor mass measured 50 by 40 by 40 mm., and seemed to consist of three coalescent nodules, one hemorrhagic, one largely

necrotic, and a third more fleshy tissue without necrosis. In the left flank is another nodule 6 mm. in diameter, which seems to be a cystic lymph gland without neoplastic involvement. The lower lobe of the left lung is riddled with minute tumor nodules, but there are no metastases elsewhere. The large tumor of the mammary gland is found to consist of two parts; one, the larger, is a typical hemorrhagic cystic carcinoma with no noticeable peculiarities. This corresponds to the hemorrhagic part of the tumor as described above. Adjacent to this is a tissue composed solely of spindle cells, with but little formation of fibrillar substance, which evidently constitutes the greater part of the tumor. These cells grow up to the connective tissue surrounding the carcinoma, but do not invade it although not sharply walled off; there is none of this tissue found within the carcinoma itself. Towards the center of the spindle-cell growth the cells are less numerous and there are various degrees of degeneration and fibrosis. Mitotic division seems to be less abundant than amitotic in this part of the tumor. The sarcoma invades the adjacent muscle freely, but the regional lymph glands are not affected. In the lungs are many metastatic nodules which exhibit only the carcinomatous elements.

These two tumors have no intimate connection with one another, and we find no evidence to indicate that one bore any etiological relation to the other. We can merely say that we have a carcinoma and a sarcoma arising side by side in the mammary gland; consideration of their relation would be only speculation. This case somewhat resembles the one reported by Haaland (16).

TYPES OF SARCOMAS REPRESENTED

Spindle-cell sarcomas were by far the most abundant, constituting 47, or a little over one-half of the tumors. These illustrated all possible types, from the fibrosarcomatous, or "desmoid" growths (fig. 4), to the very large cell sarcomas with uninuclear and small multinuclear giant cells (fig. 5). It seems unnecessary to describe the characters of these tumors in detail,

for they correspond in structure absolutely with the sarcomas occurring in man and given the same designations. Ten were diagnosed as round-cell sarcomas, ten were called "polymorphous"-cell sarcoma, and three seemed best described as oval-cell. Among the "polymorphous"-cell sarcomas were three or four which contained so many extremely large uninuclear cells that they might be entitled to the designation of giant-cell sarcoma. There were no sarcomas showing typically the myeloid type of giant cells, nor have we observed multiple myelomas or melanotic sarcomas; it may be recalled that Haaland reported a melanoma arising in the ear of a mouse.

A few of the sarcomas show a perivascular arrangement that resembles the structure commonly diagnosed as a perithelial sarcoma or hemangiosarcoma; three tumors showed this character conspicuously (fig. 6), arising one each from the scrotum, mammary gland and omentum. Two sarcomas only seemed to be entitled to be called alveolar sarcomas, and these were not very typical examples of this type of growth.

Twelve of the growths were osteosarcomas or chondro-osteosarcomas, and these presented many interesting features. They were usually very large and hard, and produced more extensive metastasis than any other type of neoplasm that we have found in mice. Because of their peculiarities they seem worthy of special consideration.

They occurred equally in both sexes, six in each. Only three failed to show metastases recognizable with the naked eye, an incidence of 75 per cent, which is, as far as we have read or observed, unequalled in any other type of neoplasm in mice if we exclude the lymphomatous new growths as of doubtful character. The distribution of the metastases was extensive—they occurred six times in the lung, five in the liver, twice each in mediastinum and diaphragm, once each in spleen, lymph glands, and knee (the last possibly being an independent primary tumor). Five arose from the hind leg, apparently the femur, with pathological fracture in three; four from the subcutaneous tissue or mammary gland, and three appeared to start from the chest wall or ribs. In general, they correspond closely to the case

described by Murray, in which the tumor arose in the mammary region, and, as he remarks, they resemble the osteoid tumors found not infrequently in the mammary gland of the dog. Haaland, as before mentioned, also observed a similar tumor arising in the thigh of one of his mice, and in the spinal column of another.

An idea of the extent of these osteosarcomas and their metastases may be obtained from the following abstracts of the autopsy records of two cases:

9554. Female. Left femur spontaneously fractured near the hip, February 25, 1915; when the animal died on March 1 the left hind foot was necrotic. A tumor mass measuring 20 by 16 by 16 mm. arose apparently at the hip and spread up into the pelvis and retroperitoneal tissues; color, white; consistency, hard. It had displaced the left ureter which lay ventral to the tumor and passed diagonally across it. It also involved the peritoneum and the body wall; in the thigh it extended nearly to the left knee. The urinary bladder was distended and the left kidney cystic. The right kidney was buried beneath the right lobe of the liver, which was adherent to the intestines and to the parietal peritoneum. The liver was about twice the normal size, riddled with secondary tumor nodules from the pinpoint to 3 mm. in diameter. There was also an abscess about a tapeworm which extended into the common bile duct and intestines. The spleen was enlarged and contained in its upper surface a tumor nodule 1 mm. in diameter. Both lungs were riddled in all parts by secondary tumor nodules.

7983. Male. At the base of the ribs on the left side, and involving also the adjacent abdominal wall, was an extremely hard mass of tissue about 8 mm. in diameter, nearly spherical, white, with blood vessels passing over its surface. Adjacent thereto and further dorsal was a second mass of about the same size, somewhat less hard, reaching almost to the dorsal midline. Attached to the upper surface of the diaphragm was a similar, extremely hard mass, about 2 mm. in diameter. The upper part of the right lung was riddled with small hard tumor nodules, with a few softer nodules in the lower lobe. There were also

tumor nodules in the posterior mediastinal tissues about the hilum of the lungs.

The histology of these tumors varies considerably. In some the primary growth and metastases are all composed of typical osteoid tissue, with trabeculæ of hyaline osteoid tissue showing greater or less calcium deposition in the centers and more cellular peripheries, often with fat tissue between the trabeculæ. Some show much calcification, some little, and true cartilage and bone are always scanty. In most instances the character varies considerably in different parts, there being areas of spindle or polymorphous cell sarcoma tissue with little or no osteoid tissue; necrosis, hemorrhage and mucoid degeneration are common. The metastases also vary in structure, but usually show a more pure type of osteoid sarcoma with a radiating structure and lobulated periphery, forming a striking picture (fig. 7).

In two cases, the livers of these mice showed most remarkable changes, consisting of isolated islands of fatty tissue, in which no liver cells remained in recognizable form, even as fatty metamorphosed liver cells (fig. 8). At first glance the impression is obtained that these are islands of simple fatty infiltration of the liver, but it is soon noted that there are generally more nuclei present than is usual in such a condition, and that these are not the ordinary flattened liver cell nucleus of the signet ring type of cell. Furthermore, the mouse liver is not much inclined to fatty infiltration of the type common in the liver of man and many other animals. In some islands it is at once apparent that this fatty tissue is replacing tumor nodules in the liver. In these earlier stages the number of round cells is so great that, admixed with the fat spaces and capillaries, the picture is that of fatty marrow associated with the osteoid tissue of the metastatic growth (fig. 9). The course of events in these two cases seems best interpreted as follows: The earliest stage of the metastatic growths is simply that of an osteoid sarcoma, about which there soon is laid down a collection of lymphoid cells resembling those of the marrow. In this cellular tissue fatty areolar tissue appears, increasing in amount with atrophy of the tumor tissue until all that is left is an island of fatty tissue resembling fatty

marrow. Later the lymphoid elements disappear still further, leaving merely a well defined area of fatty areolar tissue surrounded by practically intact liver tissue.

Such a process we have never seen described in connection with tumors, and it would be less comprehensible if we did not know that any tissue that becomes calcified is likely to subsequently develop not only true bone tissue but also true marrow tissue, evidently carrying on the functions of marrow. By this process we may come to have marrow tissue in calcified heart valves, sclerosed vessels, calcified contents of the eye, and healed pulmonary lesions. The difficulty of this explanation for our liver nodules lies in the complete disappearance or absence from some of the fatty areas of all tumor tissue, and the fact that the fatty marrow-like tissue surrounds the osteoid tissue instead of being enclosed by it as an ordinary pathological marrow formation. However, we have been unable to find a better explanation which agrees with the fact that this hepatic lesion has been found only in these two cases of osteosarcoma, and that the fatty areas are associated with metastatic growths in many instances.

MIXED TUMORS

Besides the two instances described above in which carcinoma and sarcoma seemed to arise side by side, but apparently independently, we have three cases in which the structure of the tumor suggests a combination of epithelial and sarcomatous elements. These are not included in our list of 87 sarcomas.

3383. A female mouse with two areas of tumor growth in the mammary gland. Death from amyloidosis and hypertrophy of the heart. One tumor on the left side slightly ulcerated, measuring 22 by 15 by 9 mm., was a tubular carcinoma, without anything out of the ordinary about its structure. The other, 12 mm. in diameter, also ulcerated, in the right inguinal region, consisted of two distinct elements and its structure varied in different parts. In some areas the growth resembled in most respects a tubular carcinoma, with small cystic spaces lined with low cuboidal or flattened epithelium; but even in these areas

most resembling carcinoma there are accumulations of spindle cells between and surrounding the tubular structures. These spindle cells are of medium size, with a nearly solid, deep staining nucleus, and but little discernible cytoplasm. In the greater part of the tumor these spindle cells are the predominant element, forming accumulations of a partially concentric structure and separated by a loose connective tissue which is not well defined. Even in the latter areas, tubular structures occasionally appear. The character of the cellular elements, and especially their relation to each other, recall the embryonal adenosarcoma of the kidney, which this tumor resembles closely in microscopic features, a resemblance observed by all the pathologists who have examined it. However, in this mouse the kidneys were free from tumor growth, and there were no metastases of the mammary gland tumors. Furthermore, we have never seen an embryonal adenosarcoma of the kidney in mice, and know of no such tumor having been reported in the literature. In favor of the sarcomatous, or at least the mesodermal origin of this tumor, is the presence, in both the stroma and some of the tumor nodules, of a type of mucoid or myxomatous degeneration similar to that found in connective tissues. This tumor agrees in many respects with the standards set for endotheliomas, which Hansemann maintains is the proper designation for most of the mammary gland tumors of mice.

3413. A female mouse (related to 3383 in that its aunt, 529, was a second cousin of 3413), died of acute pulmonary infection in pregnancy. In the right anterior mammary gland was a 3 mm. nodule, hard, white, with attached cyst; it was not ulcerated. On the right side anterior to the posterior mammary gland is another small white nodule, about 3 mm. in diameter, but not cystic. There are no other tumor growths or metastases, and the kidneys show no abnormalities.

The anterior growth consists of a mass of oval shaped cells, closely packed together, except for the presence of several cystic cavities. It is distinctly within the mammary gland and has evidently grown by expansion rather than infiltration, although there is evident invasion of the capsule by tumor cells in many

places. The cells are somewhat smaller and less elongated than the cells in the tumor of 3383, and the nuclei have less dense chromatin. Occasional mitotic nuclei are seen. The cysts have no definite lining, beyond that furnished by the tumor cells which lie at the surface without forming a distinct membrane. Nevertheless, in the substance of the tumor are occasional collections of cells, not essentially different from the spindle or oval cells of the tumor, which are grouped to form more or less complete tubules. These have no basement membrane, but lie in contact with the spindle cells in such a way as to suggest the so-called "rosettes" of retinal gliomas or neurocytomas.

The smaller nodule has exactly the same structure as the tumor in 3383 (fig. 10). Hence these two tumors in 3413 are somewhat different from each other, but both have the same fundamental character of showing elements resembling those of a spindle-cell sarcoma and tubular structures suggesting an adenomatous nature.

11939. A female mouse had a tumor in the left subaxillary region that seemed to be one of the usual mammary gland carcinomas. It measured 20 by 18 by 18 mm. The lungs, mediastinal tissues, and chest wall were riddled with small tumor nodules.

Microscopically, the primary tumor consists of varying structures. In large part it is composed of solidly packed cells arranged in alveoli, into which connective tissue fibrils do not penetrate, thus resembling carcinoma, especially in that the cells nearest the stroma are frequently cuboidal. The cells are of medium size, polyhedral, with deep staining but somewhat vesicular nuclei, and a moderate amount of finely granular cytoplasm. The stroma is extremely scanty, and the alveoli for the most part seem to have but little between them except blood channels in which the walls show an endothelial lining only in some places; to a large extent these blood spaces seemed to be composed only of tumor cells. The alveolar tissue with polyhedral cells passes in many places without observable transition into masses of spindle cells, which, except in shape, resemble the polyhedral cells. This latter sort of tissue constitutes about

half the primary tumor, in some fields being practically pure, while other areas of equal size show only the alveolar structure, although most of the tissue shows a mixture of both types. In the spindle-cell areas the blood channels are especially numerous, and seem to be composed solely of tumor cells, thus resembling a sarcoma. Mitotic figures are very numerous in all parts, and the characteristic multipolar and asymmetrical mitoses of cancer are abundant. The tumor infiltrates freely, and shows some central necrosis.

The numerous metastatic growths show both types of tissue. In the lungs, they are mingled in about the same proportion as in the primary growth; in the mediastinum, the spindle-cell type predominates, although some cuboidal- and polymorphous-cell elements appear; in the chest wall, where the ribs are eroded by tumor tissue, the cells are even more of the spindle cell type.

An interpretation of such a tumor as this is difficult. It corresponds to the growths sometimes described as carcinoma sarcomatodes or as sarcocarcinoma, but it is difficult to be certain that both cells are, after all, not merely modifications of cells of one origin. Ewing (17) has recently taken this position, and Dr. E. R. Le Count, who examined the sections from this case, states that he would prefer to defend the position that the growth is entirely carcinomatous than that is consists of both sarcomatous and carcinomatous elements. Dr. Ewing also holds the same view after examining our sections.

MEDIASTINAL "LYMPHOSARCOMAS"

An interesting type of growths has been found arising in the mediastinum of eleven mice in this series, which seems to correspond closely to the characteristic neoplasms observed in the same location in man, and usually designated as lymphosarcoma. Similar mediastinal neoplasms have been described in mice by others. Thus, Tyzzer (18) described four tumors arising in the thymus among ten cases of lymphomatous tumors, and these correspond closely to our material in many respects. On account of the great uncertainty concerning the nature of the lympho-

matous growths in mice, and as a special study of them as a group is under way, they are not included in this series of sarcomas, and will be described only briefly.

Five were in female mice, six in males. The typical cases show the superior mediastinal space filled with a white, yellowish white or slightly pinkish tissue, soft, and apparently arising from the site of the thymus. Sometimes the growth seems to be encapsulated but expansile, crowding the lungs apart, pushing down or spreading over the heart; less often it is infiltrative and adherent to the adjacent chest wall, pericardium and other structures. In all cases the growth surrounds and infiltrates the trachea, and spreads along the bronchi and great vessels into the hilum of the lung where it forms sheaths of tissue about these structures for some distance into the lung. Usually the pericardium, mediastinal fatty tissues, esophagus, and often the myocardium, are infiltrated by the tumor cells. In contrast to leukemia and pseudoleukemia, which often also cause lymphoid growths in the mediastinum, the spleen, liver, kidneys, and the systematic lymph glands do not exhibit lymphoid hyperplasia or lymphomatous nodules; where such a diffuse infiltration does occur, we have not included the case, as the distinction from pseudoleukemia is too difficult. However, leukemia and pseudoleukemia, although they cause some lymphoid hyperplasia about the bronchi and great vessels of the lung, we have not found to result in the formation of such great cellular masses as are seen in these mediastinal growths. In many respects these resemble the "thymomas" as described by Ewing (19) in a recent article, especially as to their origin which is apparently from the thymus, or at least from its site.

Microscopically these tumors are all composed of dense masses of round cells, but there are considerable variations in the types of cells included under this description. In some cases the cells are much larger than lymphocytes with a small amount of cytoplasm staining well with eosin, and a relatively pale, semivesicular nucleus. In others the cells approach more closely to the lymphocytes, but always larger than normal lymphoid cells. Intermediate types occur, and there may be some admixture of

different types of cells, although usually one type of cell predominates greatly. The cells are too closely packed together for any definite arrangement although a delicate reticulum divides them up into pseudo-lobules in some places. Extensive infiltration into all adjacent tissues is always seen, although mitotic figures are scanty in most cases; they are most abundant in the larger cell types. The cells invade freely the trachea, esophagus, pericardium, myocardium, and especially the mediastinal areolar tissue; in the lungs they spread luxuriantly along the bronchi and large vessels but do not infiltrate much into the adjacent lung tissue. Often large hyaline cells with one or more nuclei are present, resembling certain cells described in tumors arising in the same location in man. In two only were structures found that seemed to be remnants of Hassell's corpuscles, but in one of these this tissue was abundant with some "cholesterol slits" and other evidences of degeneration. Necrosis was seldom seen in these tumors, and then not extensive, nor was hemorrhage common.

METASTASIS

We have not yet tabulated the frequency of metastasis of carcinoma in the Slye stock, but Murray reports about 50 per cent of spontaneous carcinomas with metastasis in his material. In the 87 mice of this series 23 showed metastatic tumors, or 26.4 per cent. The height of this figure is largely caused by the osteosarcomas, which accounted for 9 of the 27. Taking up the type of sarcoma with the number giving rise to metastases, we have

Osteosarcomas	9 of 12 or 75
Spindle-cell	6 of 47 or 13
Polymorphous-cell	3 of 10 or 30
Oval-cell	0 of 3 or 0
Round-cell	4 of 10 or 40
Alveolar	1 of 2 or 50
Angiosarcoma	0 of 3 or 0
Total	23 of 87 or 26.4

We find that, as in human sarcomas, the spindle-cell sarcomas are much less likely to produce metastases than the

polymorphous-cell or round-cell types, and those spindle-cell sarcomas that produced metastases were mostly of the larger cell types (fig. 11). The fibrosarcomas rarely produce metastasis. In some cases, especially with the osteosarcomas, multiple metastases were found. The distribution of the metastases was as follows: Lung, 13; liver, 10; lymph glands, 5; mediastinum, 3; chest wall, 3; spleen, 2; pleura, 2; diaphragm, 2; retroperitoneal, 2; kidney, 1. These figures refer solely to the number of times these organs were found the seat of metastasis, in many cases there having been many secondary growths in a given organ. It will be noted that the sites of metastasis correspond closely with what is observed in human sarcoma, the lung and liver being most often involved, and then the lymph glands.

ETIOLOGY

As with human tumors, mouse sarcomas frequently arise at the site of a trauma. This has been observed in eleven of this series. It is, of course, evident that we have no knowledge of how many of the other mice had received injuries at the point at which they subsequently developed a sarcoma, for the life of a mouse is beset with many accidents and deeds of violence. Especially among the males, wounds are often received in fighting. In the eleven cases in which the relation was clear, the injury was noted and afterward the sarcoma made its appearance at this point, or else an early tumor was observed at the site of a scar from some old injury. A particularly good example is furnished by case 3117, previously described under the heading of "multiple primary sarcoma." This mouse was bitten on the back and genitals, and so severely wounded that it was taken to the "hospital" to recover. There, while under observation, two typical spindle-cell sarcomas arose at the site of these wounds.

The influence of heredity in determining the occurrence of sarcoma in the site of old wounds has been especially noted in this series, and found to be important. This is a large subject, and will constitute by itself a separate paper, and, hence, will not be discussed here. Also the relation of age to tumor forma-

tion requires more detailed study than we have yet been able to give it, and is reserved for future consideration. We have observed no relation of the sarcomas to any particular form of infection or inflammation. In not a single instance have we observed any parasites in or about the sarcoma. This is of particular interest in view of the relation of liver parasites to sarcoma of the liver in rats (20). Our mice often have tapeworms filling up the bile ducts and leading to extensive abscesses of the liver, but we have never observed either sarcomas or liver adenomas arising in these lesions. There seems to be no relation between these sarcomas and the leukemias; at least in this series we had only two cases in which sarcoma co-existed with leukemia and none with pseudoleukemias. The tendency to the co-existence of tumors is, however, quite marked, as with all other types of spontaneous mouse tumors yet studied, and this may be interpreted as the existence of a high natural susceptibility to the formation of neoplasms in the affected animals. It is certain that the more highly cancerous the ancestry of mice, the more likely they are to have multiple independent spontaneous tumors.

SUMMARY

In a series of 12,000 autopsies on the bodies of mice dying at all ages, either from natural causes or in a relatively small proportion from accident, there were found 87 mice with neoplasms meeting all the criteria of sarcoma. These do not include any growths of the character of lymphosarcomas, because of the recognized uncertainly of the nature and diagnosis of these neoplasms; also we have excluded eleven cases of characteristic mediastinal tumors, arising at the site of the thymus and infiltrating the lungs. Tumors of the testicle, adrenal, ovaries and kidneys of "mesothelioma" character have also been omitted.

Spindle-cell sarcomas constitute over half the tumors, there being 47 of all types, not including 3 oval-cell sarcomas, 3 perivascular sarcomas, and two alveolar sarcomas. There were 12 osteoid sarcomas, and 10 polymorphous-cell, and 10 round-cell sarcomas. Metastasis was observed in twenty-three cases, or

26.4 per cent, the osteoid sarcomas leading with 75 per cent, metastasis occurred in only 13 per cent of the spindle-cell sarcomas. Lungs, liver and lymph glands showed most of the metastases. In all respects these sarcomas of mice correspond with the sarcomas of men, although we have found no examples of melanosarcoma, multiple myelomas, or myeloid sarcoma. In at least eleven cases the sarcomas definitely arose at the site of previous injuries. In a few instances there seemed to be two distinct primary sarcomas in the same mouse, and there were three instances in which the growths suggested a mixture of sarcomatous and carcinomatous elements. About half the sarcomas arose in the subcutaneous tissues, apparently from the mammary gland in most cases; and next in frequency from the osseous tissues. Two cases of sarcoma of the uterus were observed, the only uterine tumors of any kind observed in all the autopsies. Twenty of the sarcoma mice also had other independent tumors, lung tumors being most numerous. Two mammary gland tumors were found closely resembling in structure the embryonal adenosarcoma of the kidney of man and other animals, but without renal involvement. The influence of inheritance on the incidence of sarcoma has been found to be marked, but is reserved for further discussion.

REFERENCES

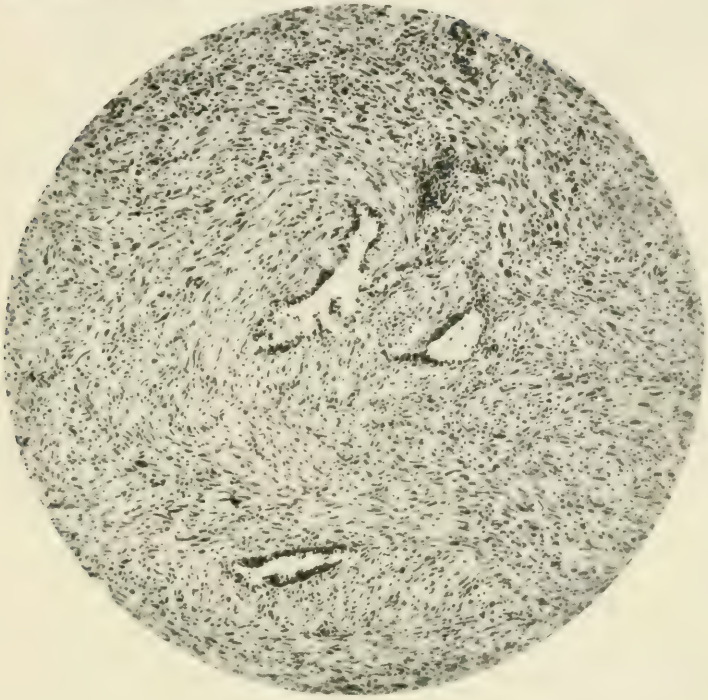
(1) Caspar, M.: Ergebn. d. allg. Path. (Lubarsch-Ostertag), 1896, iii, 2, 754.
(2) Frohner: Quoted by Caspar.
(3) McCoy, G. W.: Jour. Med. Research, 1909, xxi, 285.
(4) Woolley, P. G., and Wherry, W. B.: Jour. Med. Research, 1911, xxv, 205.
(5) Rohdenburg, G. L., and Bullock, F. D.: Jour. Cancer Research, 1916, i, 87.
(6) McCoy, G. W.: Jour. Infect. Dis., 1914, xiv, 53.
(7) Fox, H.: Jour. Path. and Bacteriol., 1912, xvii, 217.
(8) Haaland, M.: Fourth Sc. Rep., Imperial Cancer Research Fund, London, 1911, 1.
(9) Murray, J. A.: Third Sc. Rep., Imperial Cancer Research Fund, London, 1908, 69.
(10) Haaland, M.: Ztschr. f. Krebsforsch., 1907, v, 125.
(11) Jensen, C. O.: Ztschr. f. Krebsforsch., 1908-09, vii, 285.
(12) Jobling, J. W.: Monographs from the Rockefeller Institute, 1910, no. 1, 52.
(13) Tyzzer, E. E.: Jour. Med. Research, 1909, xxi, 479.

(14) Apolant, H.: Arch. f. Dermat., 1912, cxiii, 39.
(15) Haaland, M.: Fourth Sc. Rep., Imperial Cancer Research Fund, London, 1911, 11.
(16) Haaland, M.: Fourth Sc. Rep., Imperial Cancer Research Fund, London, 1911, 19 (fig.17).
(17) Ewing, J.: Jour. Cancer Research, 1916, i, 71.
(18) Tyzzer, E. E.: Jour. Med. Research, 1909, xxi, 487.
(19) Ewing, J.: Surg. Gynec., and Obst., 1916, xxii, 461.

PLATE 1

Fig. 1. Pericanalicular fibrosarcoma of the mammary gland. × 120. The tubules are lined with epithelium and are scanty.

Fig. 3. Squamous-cell carcinoma of the mouth, under which is a tissue resembling a spindle-cell sarcoma, apparently arising in the periosteum of the lower jaw. Some fields show many giant cells. The bone, in the center of the field shown, is eroded and invaded by tumor tissue. × 70.



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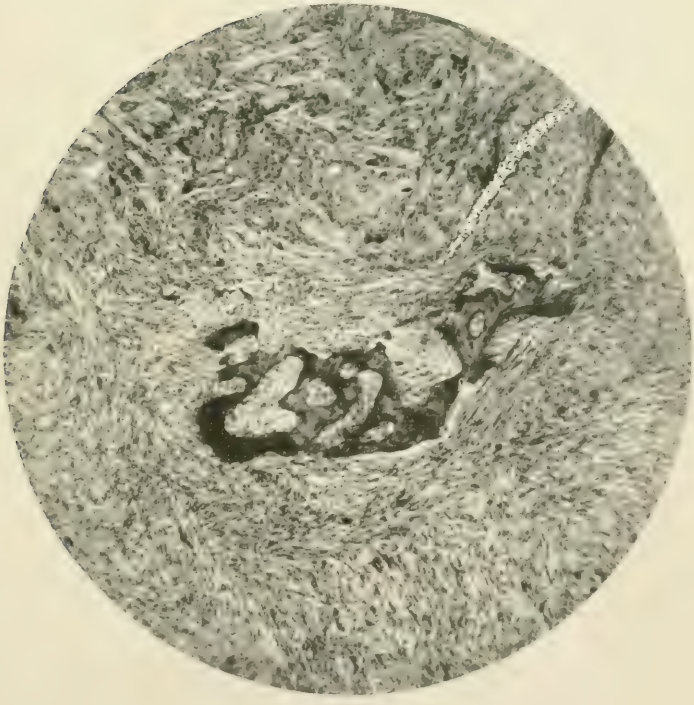

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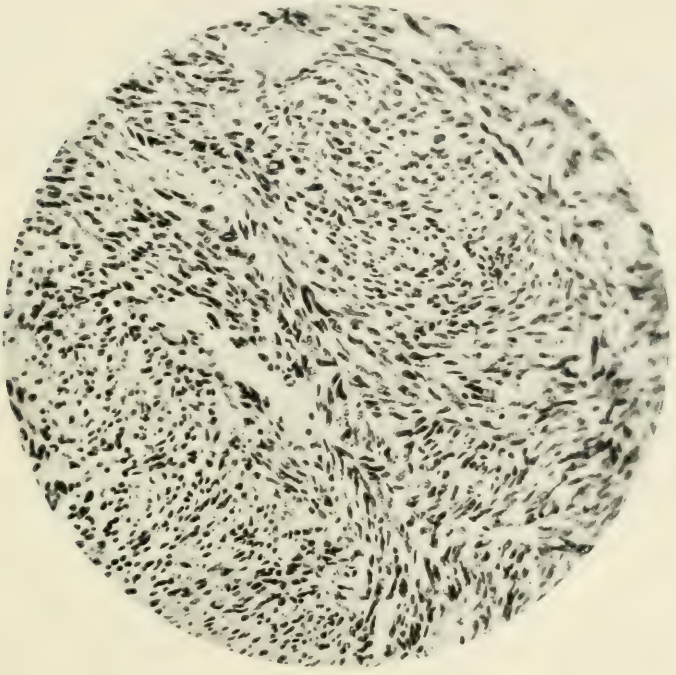
PLATE 2

FIG. 4. Spindle-cell sarcoma, arising in subcutaneous tissue, shows typical spindle-cell character, exhibited by the entire tumor. × 100.

FIG. 5. Large spindle-cell sarcoma arising in periosteum of femur, showing many small giant cells, also muscle cells infiltrated by the tumor tissue. Metastasis from this tumor in lung shown in figure 11. × 120.



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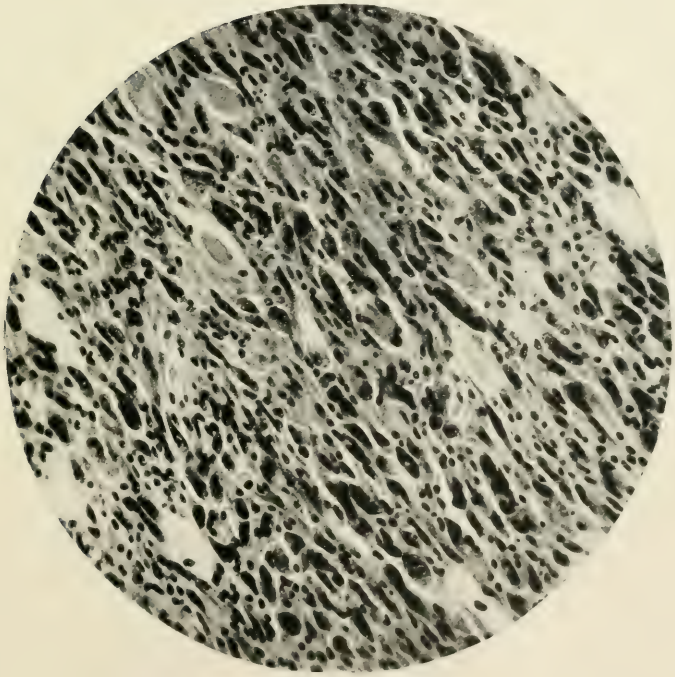

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PLATE 3

FIG. 6. Angiosarcoma arising in subcutaneous tissue. × 110.

FIG. 7. Osteoid sarcoma metastasis in liver, secondary to tumor in femur. Shows central calcification and atypical ossification, with more active cellular growth at the periphery. × 60.

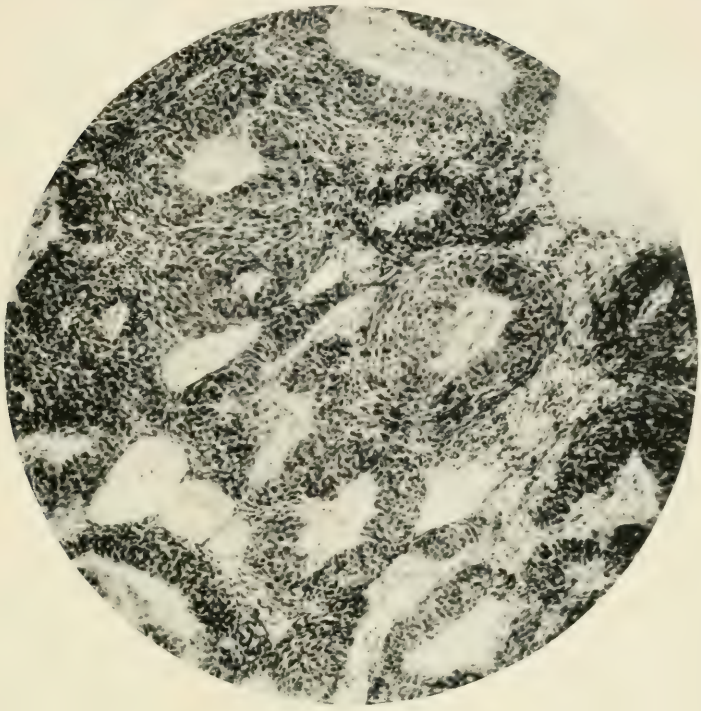

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PLATE 4

FIG. 8. Nodular areas of fatty tissue in liver of mouse with an osteoid sarcoma. In other parts of this liver tumor metastases are found. Note how well defined these areas are, and the absence of fatty changes in the liver tissue between them. × 24.

FIG. 9. Secondary osteoid sarcoma nodule in the liver, surrounded by a tissue resembling fatty marrow. All stages between those illustrated in figures 7 and 8 can be found. × 95.

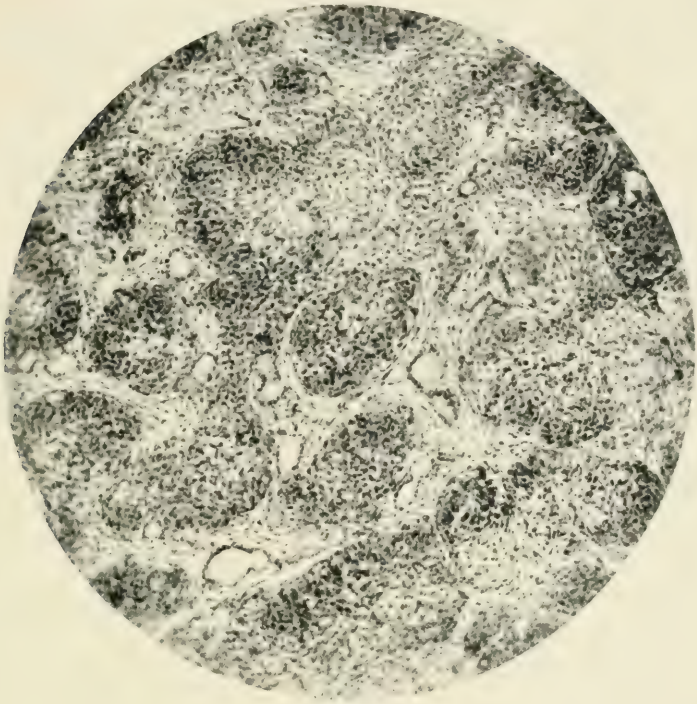
8

9

PLATE 5

FIG. 10. Mammary gland tumor resembling in histology the embryonal adenosarcomas of the kidney, although there was no renal tumor in this case. The islands of spindle cells show some mucoid degeneration. × 130.

FIG. 11. Secondary spindle-cell sarcoma in the lung, adjacent to a large bronchus. Primary growth was in the femur, and shown in figure 5. × 120.



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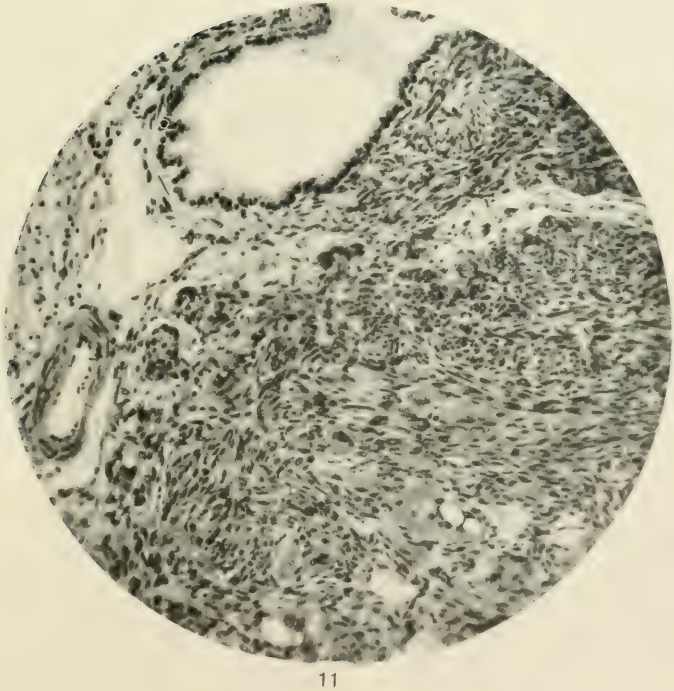

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THE INHERITANCE BEHAVIOR OF INFECTIONS COMMON TO MICE[1]

STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

Ninth Report

MAUD SLYE

From the Cancer Laboratory of the Otho S. A. Sprague Memorial Institute and the University of Chicago

Previous reports from this laboratory during the past six years have demonstrated the inheritability of spontaneous cancer in mice, both of cancer in general and of tumors of specific organs and of specific types. Some commentators have inferred, however, that the fact that the cancer tendency is inheritable has no particular bearing upon the etiology of cancer since they suppose[2] that a study of any of the common infections, such as pulmonary infections, would show similar results.

The full import of such a demonstration and its unquestionable bearing upon the nature of cancer, therefore, stands sharply out when this inheritance behavior of cancer is compared with the carefully tested inheritance behavior of the infections common to mice.

The cancers reported from this laboratory are all spontaneous, arising in the ordinary routine of mouse life without any artificial manipulation of any sort whatever. The infections reported in this paper occur also without any artificial manipulation.

It is the unbroken rule throughout these experiments to let natural causes determine the incidence of both cancer and

[1] Presented before the American Society for Cancer Research, New York, April 5, 1917.

[2] Erwin F. Smith, Further evidence that crown gall of plants is cancer, Science, 1916, xliii, 874.

infections without any interference except that of a rigorous hygiene and of a definite plan of selective breeding uniformly applied throughout the entire stock, both cancerous and non-cancerous. This method of study gives a fair basis for a comparison of the facts of the inheritance behavior of these types of disease.

It must be emphasized in the study of this comparison that there is a tremendous handicap in favor of the occurrence of infections, as follows:

1. Infections may occur at any period in mouse life from the day of birth to the date of death, with the highest incidence during the periods of infancy and of adolescence. On the other hand, cancer rarely occurs under six months in mice, and the high level of incidence centers around the period from ten to twenty months. Hundreds of mice, consequently, die within the infection age, but under the cancer limit; and there is no difficulty whatever in securing strains peppered with infections, while it is tremendously difficult to carry out a cancer strain.

2. A whole laboratory of mice can be swept off within a week by contact contraction of virulent infections, so that a constant warfare against such disease is necessary in order to preserve any stock at all. It is therefore almost impossible to keep any strain free from these infections through contact contraction, so as to be able to make any kind of a study of their inheritance behavior. Whereas, contagion experiments already described in previous publications, which have been consistently carried on in this laboratory for years, have failed to produce one case of contact contraction of cancer.

It is impossible to make any report of the behavior of tuberculosis[3] in mice because mice do not have tuberculosis. In order, however, to give every advantage to the infections in a comparison of their inheritance behavior with that of cancer, I have grouped all pulmonary infections, all intestinal infections, all uterine infections, etc., to which mice are susceptible. The test therefore, it is evident, is a test of the tendency of the in-

[3] Erwin F. Smith, loc. cit.

testines, or of the lung, or of the uterus, etc., to yield to specific infections as compared with the inheritability of their tendency to develop cancer.

In spite of the tremendous handicap in favor of the common infections peppering every strain of mice, and in spite of the great difficulty in carrying out one cancer strain to any considerable number, note the results.[4]

CHART 1

Strain 139

In this strain both parents had carcinoma of the lung, male 193, primary carcinoma, and female 158, metastatic carcinoma of the lung. The resulting strain carried 90 per cent of cancer, every member but two having primary tumors of the lung. One member, male 553, died at six months, which is under the age for the occurrence of lung tumors.

In sharp contrast with these results note the inheritance behavior of pulmonary infection shown in strain 20.

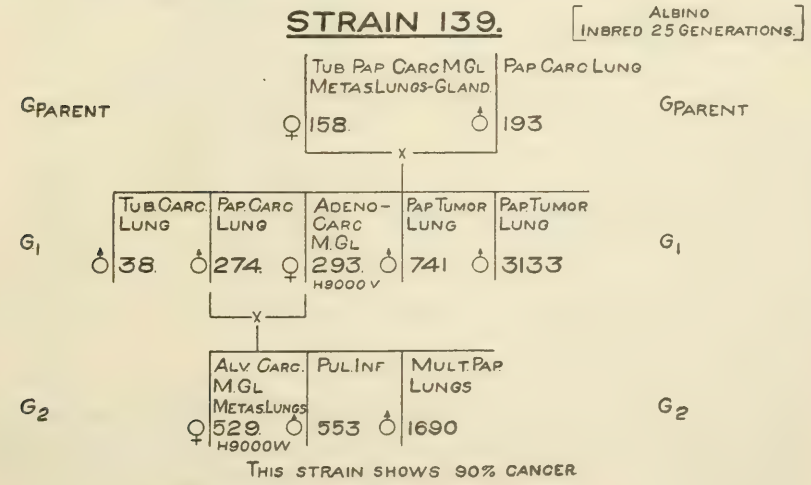


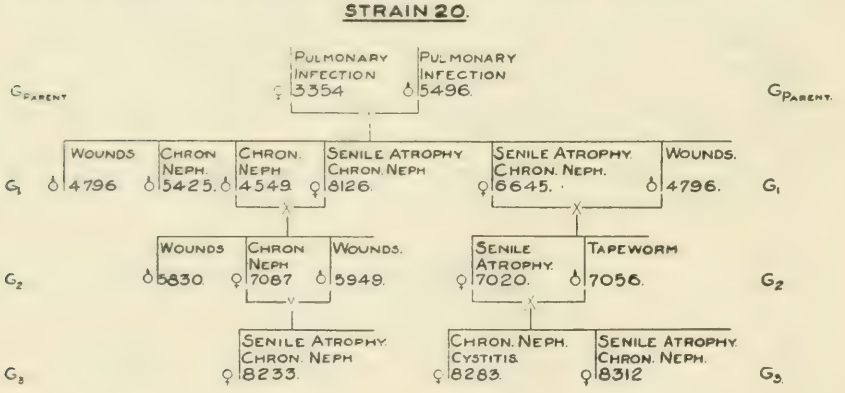
CHART 1

[4] Some of these charts have been published previously in whole or in part. They are here repeated for convenience in comparison.

CHART 2

Strain 20

This is a non-cancerous strain of the Japanese waltzing mouse in my hands several years. Although the strain is now living in the eighth generation, it has never shown an occurrence of cancer. In this strain, where both parents died of pulmonary infection, viz., female 3354 and male 5496, there has never been one recurrence of pulmonary infection. This is a striking example of the non-inheritability of pulmonary infection. The same contrast between the inheritance behavior of cancer and that of pulmonary infections is again shown in chart 3.



A NON-CANCEROUS STRAIN OF JAPANESE WALTZING MICE
BOTH PARENTS DIED OF PULMONARY INFECTION
THE RESULTING STRAIN SHOWS 0% PULMONARY INFECTION.

CHART 2

CHART 3

Strains 280 and 281

In strain 280 both parents had carcinoma of the lung, viz., male 363 and female 258. The resulting family showed 100 per cent of cancer, 80 per cent being tumors of the lung, primary or secondary.

Whereas, in strain 281, although in the first filial generation female 3270 had pulmonary infection, and in another branch of

the family in the second filial generation the parent male 2389 died of pulmonary infection, the offspring of these two mice and their respective mates never showed a recurrence of pulmonary infection.

In this same strain where both parents died of tumors, viz., female 258 with carcinoma of the lung and male 286 with adenoma of the liver, the resulting offspring showed about 20 per cent of tumors, 40 per cent of these tumors being located in the liver, and the remaining tumors being located in the lung.

CHART 4

Strain 343

In this strain the parent female 5357 died of carcinoma of the mammary gland, and the parent male 2098 died of pulmonary infection. The resulting strain shows over 58 per cent of cancer, but not one recurrence of pulmonary infection. Here where the two diseases had equal opportunity to be transmitted if both were inheritable, cancer occurred in 58 per cent of the strain and pulmonary infection not at all.

CHART 5

Strain 164. Branch IV

This strain is the result of hybridizing the highly tumorous strain 146 with a non-tumorous strain of house mice, strain 358. Although the parent female, 1236, died early without showing cancer, she evidently transmitted it for the resulting strain shows 24 per cent of tumor. Again, although the parent male in the first filial generation, male 4378, died of pulmonary infection, there was never a recurrence of pulmonary infection throughout the strain, although the family is bred out through five generations involving fifty members. This is another striking case of contrast in the inheritance behavior of cancer and that of pulmonary infection.

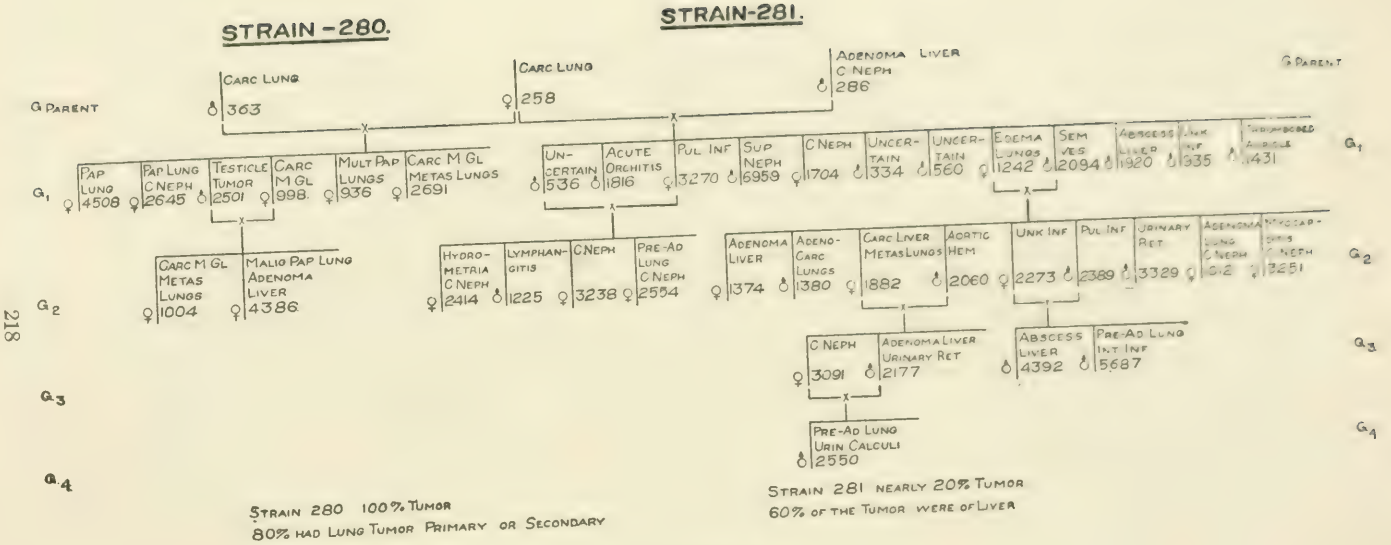
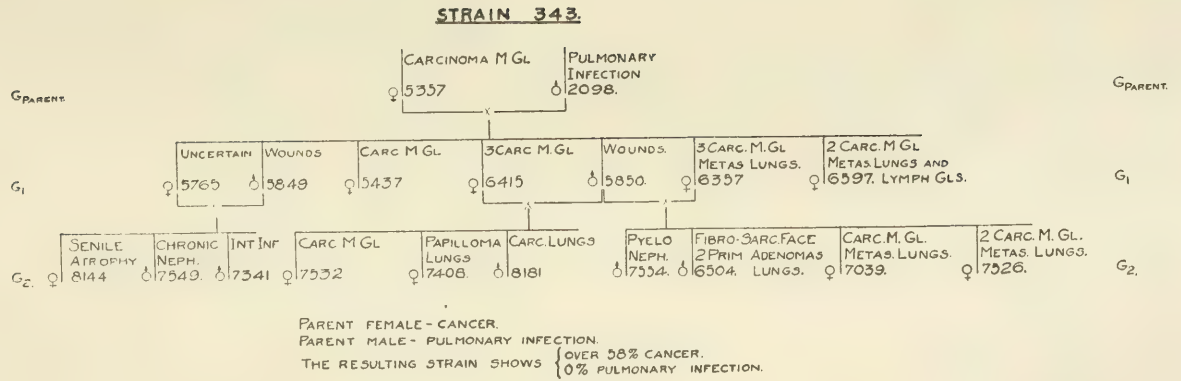
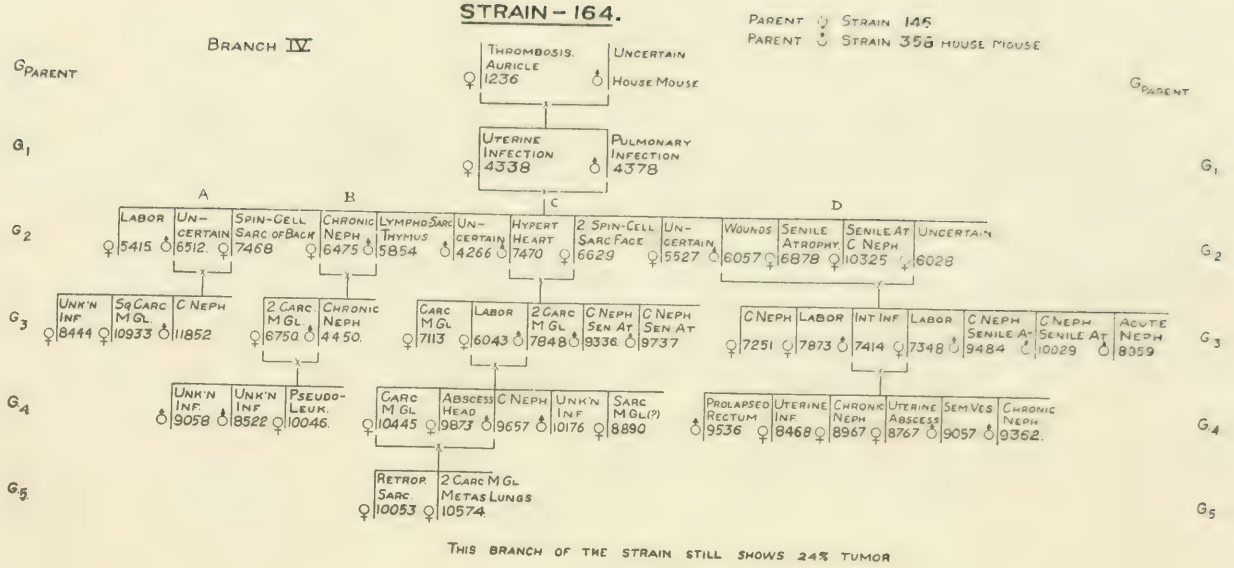

STRAIN-280.
STRAIN-281.
G PARENT
CARC LUNG ♂363
CARC LUNG ♀258
ADENOMA LIVER C NEPH ♂286
G PARENT
G1
PAP LUNG 4508
PAP LUNG C NEPH 2645
TESTICLE TUMOR 2501
CARC M GL 998
MULT PAP LUNGS 936
CARC M GL METAS LUNGS 2691
UNCERTAIN 536
ACUTE ORCHITIS 1816
PUL INF 3270
SUP NEPH 6959
C NEPH 1704
UNCERTAIN 334
UNCERTAIN 560
EDEMA LUNGS 1242
SEM VES 2094
ABSCESS LIVER 1920
UNK INF 335
THROMBOSED AURICLE 1431
G2
CARC M GL METAS LUNGS ♀1004
MALIG PAP LUNG ADENOMA LIVER ♀4386
HYDROMETRIA C NEPH ♀2414
LYMPHANGITIS ♂1225
C NEPH ♀3238
PRE-AD LUNG C NEPH ♀2554
ADENOMA LIVER ♀1374
ADENO-CARC LUNGS ♂1380
CARC LIVER METAS LUNGS ♀1882
AORTIC HEM ♂2060
UNK INF ♀2273
PUL INF ♂2389
URINARY RET ♀3329
ADENOMA LUNG C NEPH ♀3251
G3
C NEPH ♀3091
ADENOMA LIVER URINARY RET ♂2177
ABSCESS LIVER ♂4392
PRE-AD LUNG INT INF ♂5687
G4
PRE-AD LUNG URIN CALCULI ♂2550
STRAIN 280 100% TUMOR
80% HAD LUNG TUMOR PRIMARY OR SECONDARY
STRAIN 281 NEARLY 20% TUMOR
60% OF THE TUMOR WERE OF LIVER

CHART 3



PARENT FEMALE - CANCER.
PARENT MALE - PULMONARY INFECTION.
THE RESULTING STRAIN SHOWS { OVER 58% CANCER. 0% PULMONARY INFECTION.

CHART 4


STRAIN – 164.
PARENT ♀ STRAIN 148
PARENT ♂ STRAIN 358 HOUSE MOUSE
BRANCH IV
G PARENT
G PARENT
G1
G2
G3
G4
G5
THROMBOSIS. AURICLE 1236 ♀ | UNCERTAIN ♂ HOUSE MOUSE
UTERINE INFECTION 4338 ♀ | PULMONARY INFECTION 4378 ♂
A
B
C
D
LABOR 5415 ♀ | UNCERTAIN 6512. ♂ | SPIN-CELL SARC OF BACK 7468 ♀ | CHRONIC NEPH 6475 ♂ | LYMPHOSARC THYMUS 5854 ♀ | UNCERTAIN 4266 ♂ | HYPERT HEART 7470 ♀ | 2 SPIN-CELL SARC FACE 6629 ♀ | UNCERTAIN 5527 ♂ | WOUNDS 6057 ♀ | SENILE ATROPHY 6878 ♀ | SENILE AT C NEPH 10325 ♀ | UNCERTAIN 6028
UNK'N INF. 8444 ♀ | SQ CARC M GL. 10933 ♂ | C NEPH 11852
2 CARC M GL. 6750 ♂ | CHRONIC NEPH 4450.
CARC M GL 7113 ♀ | LABOR 6043 ♂ | 2 CARC M GL 7848 ♂ | C NEPH SEN AT 9336. ♂ | C NEPH SEN AT 9737
C NEPH 7251 ♀ | LABOR 7873 ♂ | INT INF 7414 ♀ | LABOR 7348 ♂ | C NEPH SENILE AT 9484 ♂ | C NEPH SENILE AT 10029 ♂ | ACUTE NEPH 8359
UNK'N INF. 9058 ♂ | UNK'N INF 8522 ♀ | PSEUDO-LEUK. 10046.
CARC M GL 10445 ♀ | ABSCESS HEAD 9873 ♂ | C NEPH 9657 ♂ | UNK'N INF 10176 ♀ | SARC M GL(?) 8890
PROLAPSED RECTUM 9536 ♂ | UTERINE INF. 8468 ♀ | CHRONIC NEPH 8967 ♀ | UTERINE ABSCESS 8767 ♂ | SEM VES 9057 ♂ | CHRONIC NEPH 9362.
RETROP SARC. 10053 ♀ | 2 CARC M GL METAS LUNGS 10574 ♀

THIS BRANCH OF THE STRAIN STILL SHOWS 24% TUMOR

CHART 5

CHART 6

Strain 151

This strain furnishes another marked instance of the failure of pulmonary infection to be introduced into the strain where one of the parents died of this infection, viz., male 250. This strain carried through four generations never produced another case of pulmonary infection. It carried, however, a low per cent of cancer.

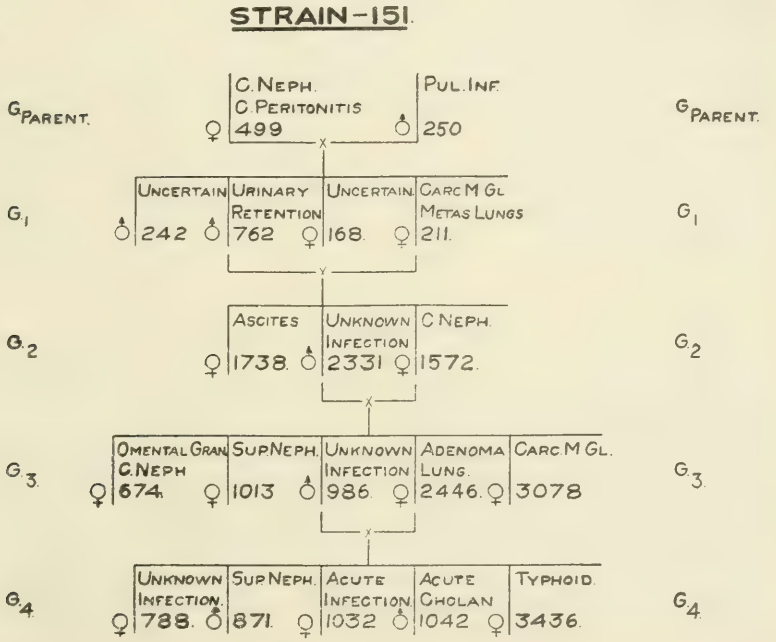


CHART 6

CHART 7

Strain 143

In this strain, also, the parent male 809 in the third filial generation died of pulmonary infection. Here again, there was never another case of pulmonary infection.

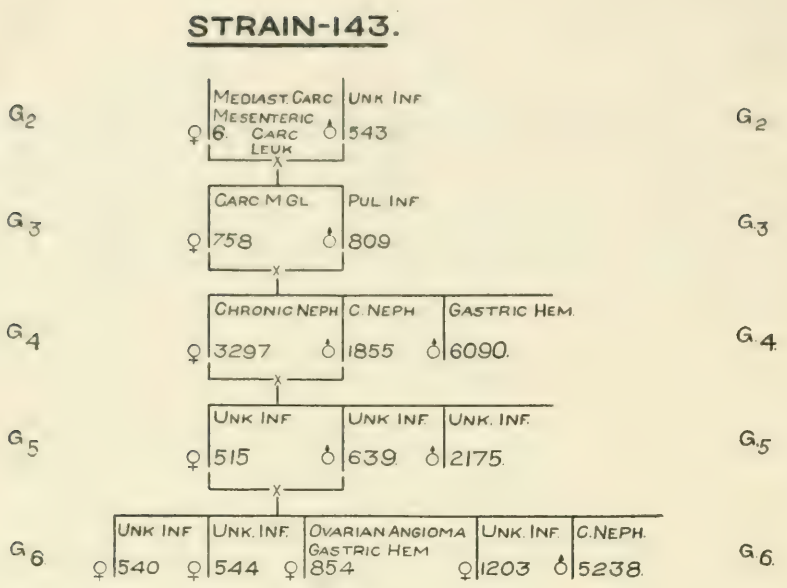


CHART 7

CHART 8

Strain 450 with ancestry

This strain shows a striking contrast in the inheritance behavior of cancer on the one hand and of pulmonary infection on the other. The parent female 5924 died of a primary carcinoma of the mammary gland and a primary carcinoma of the lung. She was the hybrid product of four strains all of which carried carcinoma in direct line and all of which showed pulmonary infection, though not in direct line, except in the case of male 250.

The parent male 5183, who died of a hypertrophied heart and intestinal infection, was the product of the hybridization of three strains all of which carried carcinoma in direct line and all of which exhibited pulmonary infection though with the exception of male 809, not in direct line.

Here, then, cancer and pulmonary infection both occurred in the ancestry on both sides of the family, but the resulting strain,

strain 450, showed over 76 per cent of cancer of the same organs and of the same types exhibited in the ancestry, but not one case of pulmonary infection.

The place of male 250 is shown in his inbred strain in chart 6, parent generation, where again there was no recurrence of pulmonary infection. The place of male 809 is shown in his inbred strain in chart 7, third filial generation, where also there was no recurrence of pulmonary infection.

These two males, 809 and 250, both with pulmonary infection, produced offspring which failed to show one case of pulmonary infection, when tested both by inbreeding (charts 6 and 7) and by hybridization (chart 8) which are the only two tests of heredity; and this in spite of the conspicuous prevalence of pulmonary infection in mice.

Contrast with this the occurrence of 76 per cent of cancer in strain 450 where cancer also was bred in on both sides of the ancestry.

CHART 9

Strain 338. Branch III

Here both parents in the first filial generation, viz., female 7899 and male 6851 died of intestinal infection contracted long after separation from their young. The resulting strain shows not one recurrence of intestinal infection, although it is carried out through five generations. Whereas, cancer, which entered in the parent generation, viz., female 5417 and male 7736 occurred in over 45 per cent of the resulting strain.

CHART 10

Strain 338. Branch I

In this branch of the family in the first filial generation, the parent female 9544 died of a carcinoma of the mammary gland, an adenoma of the liver, and a primary carcinoma of the lung; the parent male 6441, died of intestinal infection. The resulting strain shows 40 per cent of tumors, these tumors, with one exception, being carcinoma of the mammary gland, adenoma

STRAIN – 450 WITH ANCESTRY.

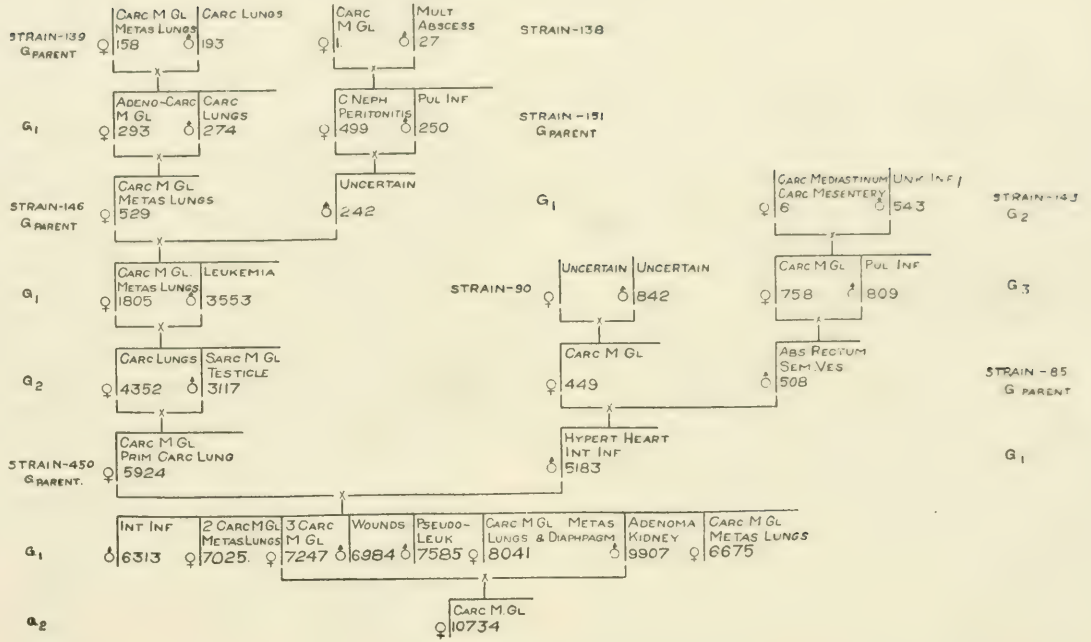
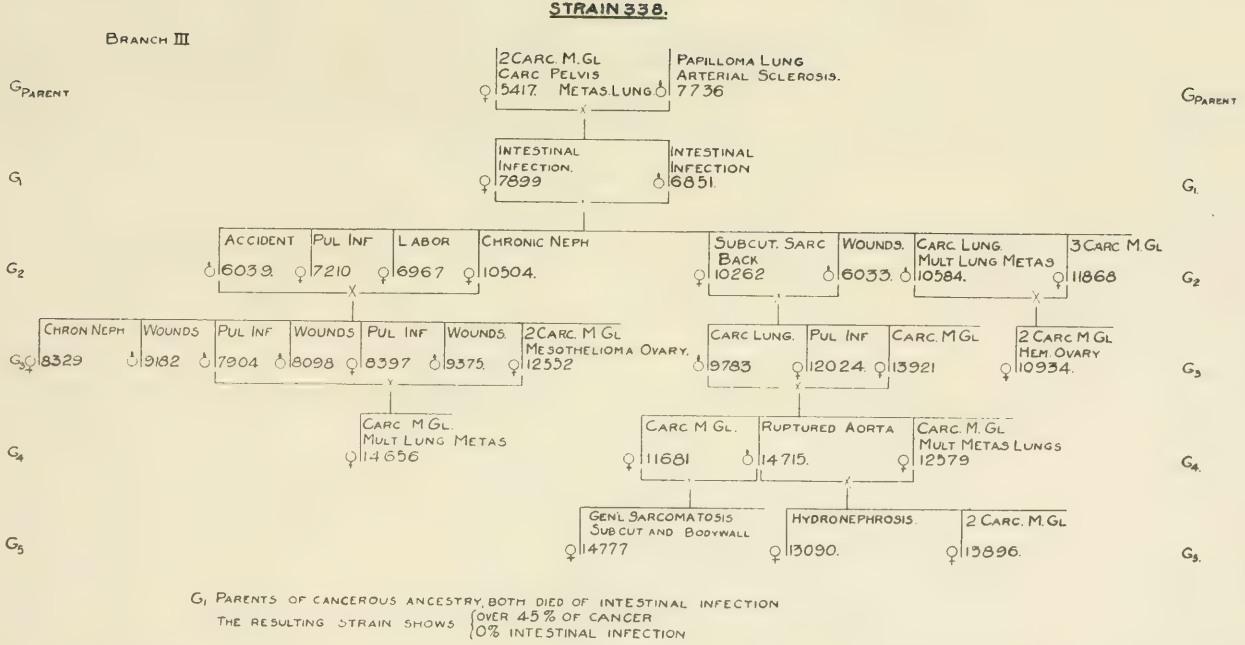


CHART 8

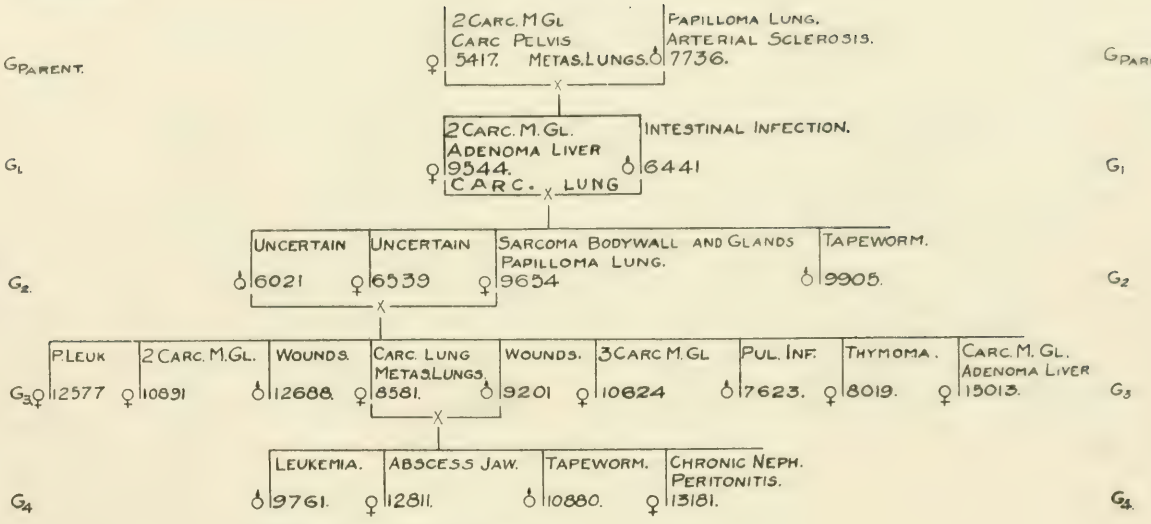


G1 PARENTS OF CANCEROUS ANCESTRY, BOTH DIED OF INTESTINAL INFECTION
THE RESULTING STRAIN SHOWS { OVER 45% OF CANCER / 0% INTESTINAL INFECTION

CHART 9

STRAIN 338.

BRANCH I.



G_1. PARENT FEMALE - CANCER.
G_1. PARENT MALE - INTESTINAL INFECTION.
THE RESULTING STRAIN SHOWS { NEARLY 39% CANCER. 0% INTESTINAL INFECTION.

CHART 10

of the liver, and carcinoma of the lung, exactly the locations and types of tumors represented in the female. Yet not one case of intestinal infection occurred in the family, although this family is bred out in four generations.

CHART 11

Strain 338. Branch XI

Here again in the first filial generation the individuals which head this branch showed on the one side carcinoma and on the other side intestinal infection, viz., female 6993 and male 6851.

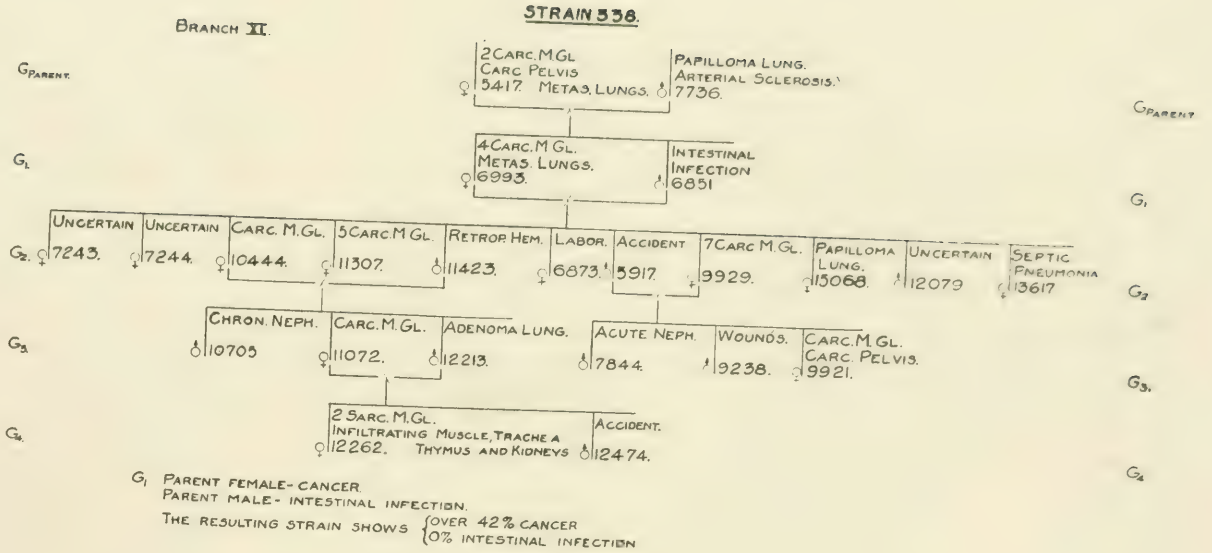
The two types of disease had, therefore, an equal chance of transmission if both were inheritable. Cancer, however, occurs in over 42 per cent, whereas not one case of intestinal infection ever appeared; and this in spite of the paucity of mouse cancers in general, and the overwhelming frequency of intestinal infections in mice.

Note the tendency in this branch to the occurrence of multiple primary tumors of the mammary gland: two in female 5417; four in female 6993; five in female 11307; seven in female 9929; two in female 12262. Note the close approximation in female 9921, filial generation 3, to the tumor production of her great grandmother, female 5417, parent generation of the branch. And in sharp contrast to this note the lone case of intestinal infection in male 6851, which left no imprint upon the strain.

CHART 12

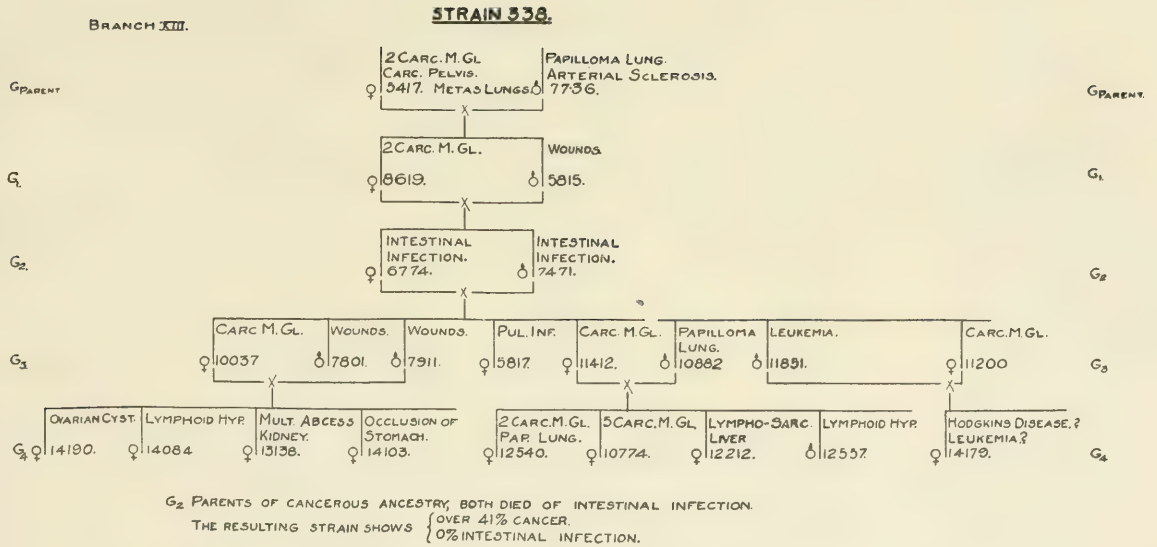
Strain 338. Branch XIII

Again, in this branch of the family, both parents of the second filial generation died of intestinal infection, viz., female 6774 and male 7471. No instance of this disease ever appeared in the resulting strain. The female of the parent generation and the female of the first filial generation, however, carried carcinoma of the mammary gland and the male of the parent generation carried papilloma of the lung. Note the recurrence of these locations and types of tumors in the resulting strain, which showed over 41 per cent cancer, 0 per cent intestinal infection.



G_1 PARENT FEMALE - CANCER.
PARENT MALE - INTESTINAL INFECTION.
THE RESULTING STRAIN SHOWS { OVER 42% CANCER
0% INTESTINAL INFECTION

CHART 11



G_2 PARENTS OF CANCEROUS ANCESTRY, BOTH DIED OF INTESTINAL INFECTION.
THE RESULTING STRAIN SHOWS { OVER 41% CANCER. 0% INTESTINAL INFECTION.

CHART 12

CHART 13

Strain 413. Branch A

In the first filial generation of this branch of the family the parent female, 7701, had carcinoma, the parent male, 6606, died of pulmonary infection. The branch carried over 33 per cent of tumors; 0 per cent of pulmonary infection.

CHART 14

Strain 413. Branch B

In branch B of this strain the introduction of intestinal infection by the first filial generation, male 6900, and the corresponding introduction of carcinoma by his mate, female 6874, produced the following results: cancer, over 22 per cent; intestinal infection, 0 per cent.

Note that in the second filial generation the parent male, 6998, died of pulmonary infection. There was no recurrence of pulmonary infection in his descendants.

Note that the introduction of sarcosporidiosis by male 14835 (second filial generation) left no imprint upon the resulting strain. This male contracted sarcosporidiosis from a female with which he was hybridized long after his isolation from the family represented in this chart.

There was shown no inherited tendency to sarcosporidiosis; no inherited tendency to pulmonary infection; no inherited tendency to intestinal infections in this strain, although all these types of disease were represented in the parentage. On the other hand, the cancer tendency appears in over 22 per cent in the resulting strain.

CHART 15

Strain 146. Branch C

Charts 15, 16 and 17 represent three lines in which branch I of strain 146 was bred out. Note here the conspicuous evidence of the segregating out of the cancer tendency and the non-cancer tendency as inheritable characters. In branch C, the

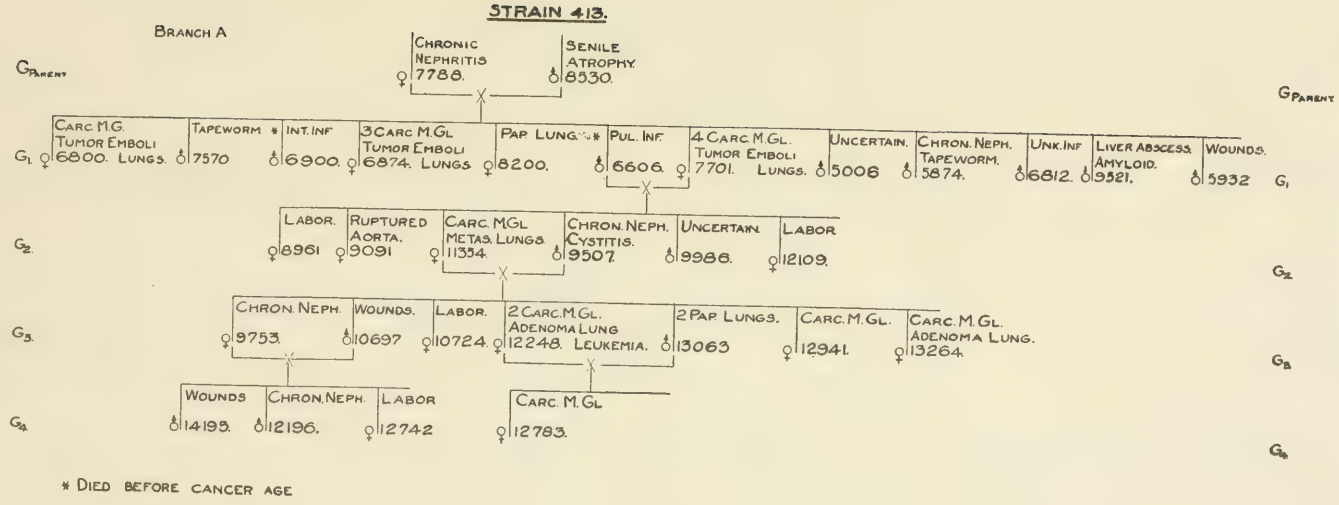
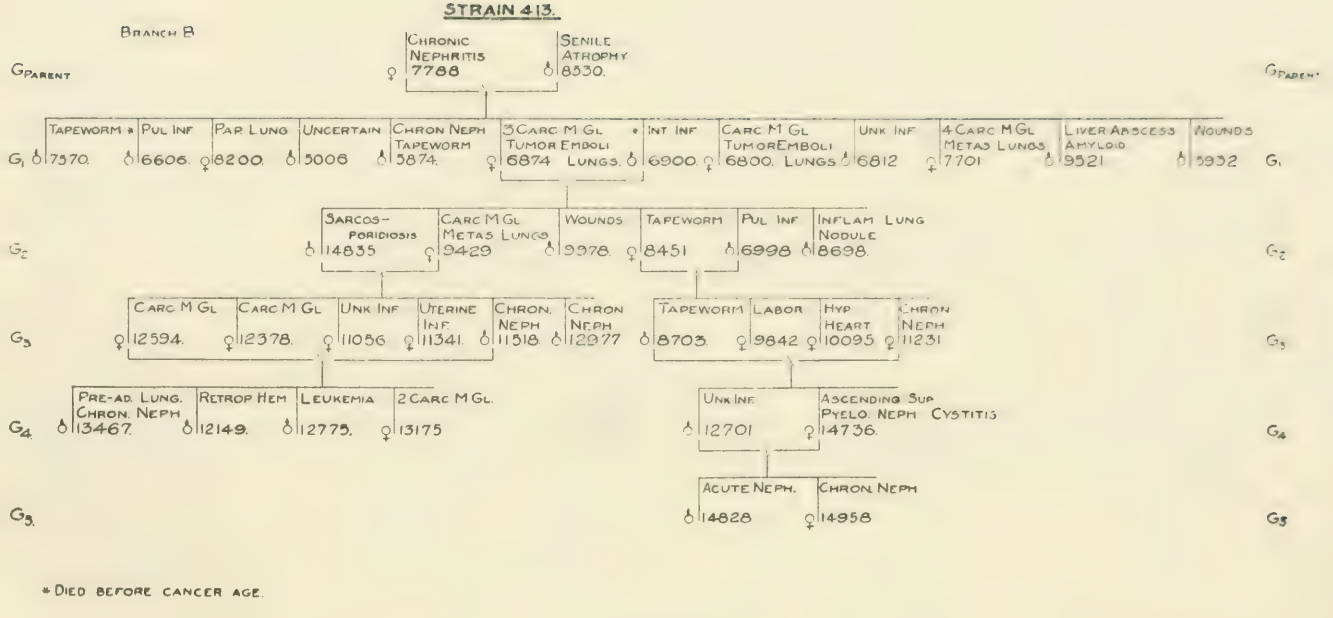

STRAIN 413.
BRANCH A
G Parent
Chronic Nephritis 7788.
Senile Atrophy 8530.
G Parent
G1
Carc. M.G. Tumor Emboli 6800. Lungs.
Tapeworm 7570
Int. Inf 6900.
3 Carc. M.Gl. Tumor Emboli 6874. Lungs
Pap. Lungs * 8200.
Pul. Inf. 6606.
4 Carc. M.Gl. Tumor Emboli 7701. Lungs.
Uncertain. 5008
Chron. Neph. Tapeworm. 5874.
Unk. Inf 6812.
Liver Abscess. Amyloid. 9321.
Wounds. 5932
G1
G2
Labor. 8961
Ruptured Aorta. 9091
Carc. M.Gl. Metas. Lungs. 11354.
Chron. Neph. Cystitis. 9507.
Uncertain. 9986.
Labor 12109.
G2
G3
Chron. Neph. 9753.
Wounds. 10697
Labor. 10724.
2 Carc. M.Gl. Adenoma Lung 12248. Leukemia.
2 Pap. Lungs. 13063
Carc. M.Gl. 12941.
Carc. M.Gl. Adenoma Lung. 13264.
G3
G4
Wounds 14195.
Chron. Neph. 12196.
Labor 12742
Carc. M.Gl. 12783.
G4
* Died before cancer age

Chart 13



* DIED BEFORE CANCER AGE.

CHART 14

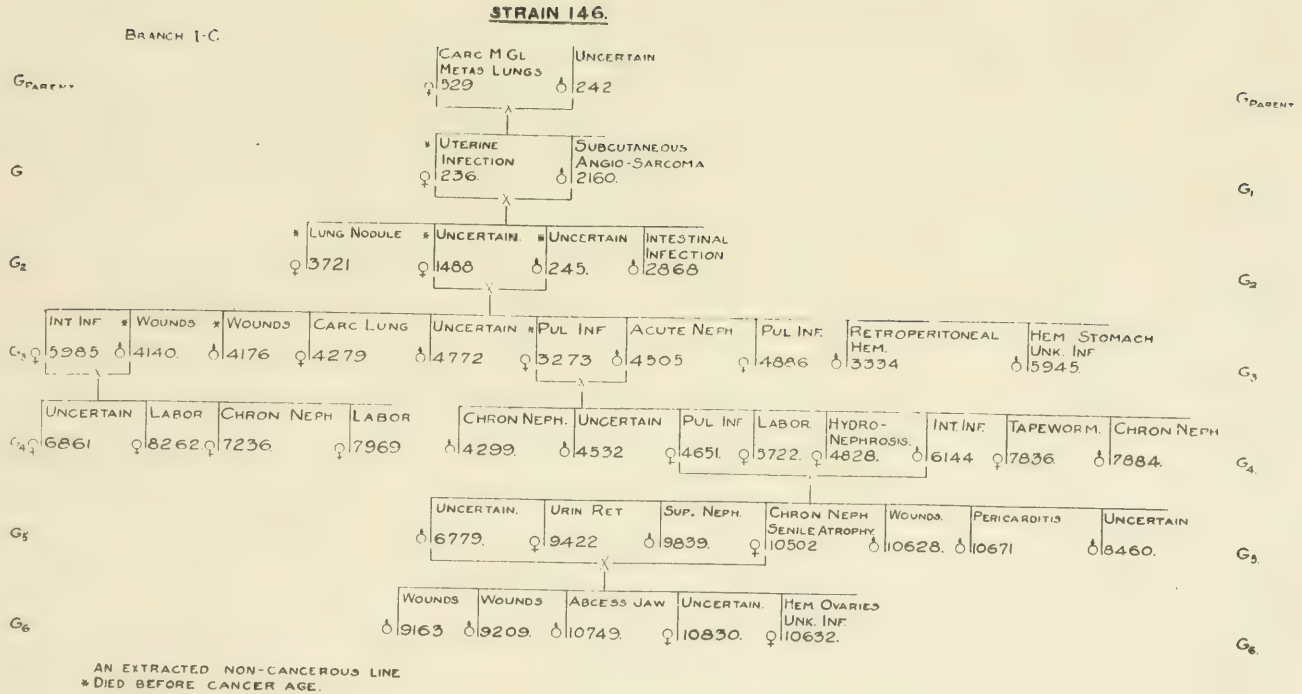

STRAIN 146.
BRANCH 1-C.
G PARENT
CARC M GL METAS LUNGS ♀529
UNCERTAIN ♂242
G PARENT
G
* UTERINE INFECTION ♀236.
SUBCUTANEOUS ANGIO-SARCOMA ♂2160.
G1
G2
* LUNG NODULE ♀3721
* UNCERTAIN. ♀1488
* UNCERTAIN ♂245.
INTESTINAL INFECTION ♂2868
G2
G3
INT INF ♀5985
* WOUNDS ♂4140.
* WOUNDS ♂4176
CARC LUNG ♀4279
UNCERTAIN ♂4772
* PUL INF ♀3273
ACUTE NEPH ♂4505
PUL INF. ♀4886
RETROPERITONEAL HEM. ♂3334
HEM STOMACH UNK. INF ♂5945.
G3
G4
UNCERTAIN ♀6861
LABOR ♀8262
CHRON NEPH ♀7236.
LABOR ♀7969
CHRON NEPH. ♂4299.
UNCERTAIN ♂4532
PUL INF ♀4651.
LABOR. ♀5722.
HYDRO-NEPHROSIS. ♀4828.
INT. INF. ♂6144
TAPEWORM. ♀7836.
CHRON NEPH ♂7884.
G4
G5
UNCERTAIN. ♂6779.
URIN RET ♀9422
SUP. NEPH. ♂9839.
CHRON NEPH SENILE ATROPHY ♀10502
WOUNDS. ♂10628.
PERICARDITIS ♂10671
UNCERTAIN ♂8460.
G5
G6
WOUNDS ♂9163
WOUNDS ♂9209.
ABCESS JAW ♂10749.
UNCERTAIN. ♀10830.
HEM OVARIES UNK. INF. ♀10632.
G6
AN EXTRACTED NON-CANCEROUS LINE
* DIED BEFORE CANCER AGE.

CHART 15

line in which it is bred out through female 3273 and male 4505 (second filial generation) is absolutely cancer free, an extracted non-cancerous line.

CHART 16

In branch B there arose an extracted cancerous line, every mouse of which that lived to more than barely six months of age, died of cancer.

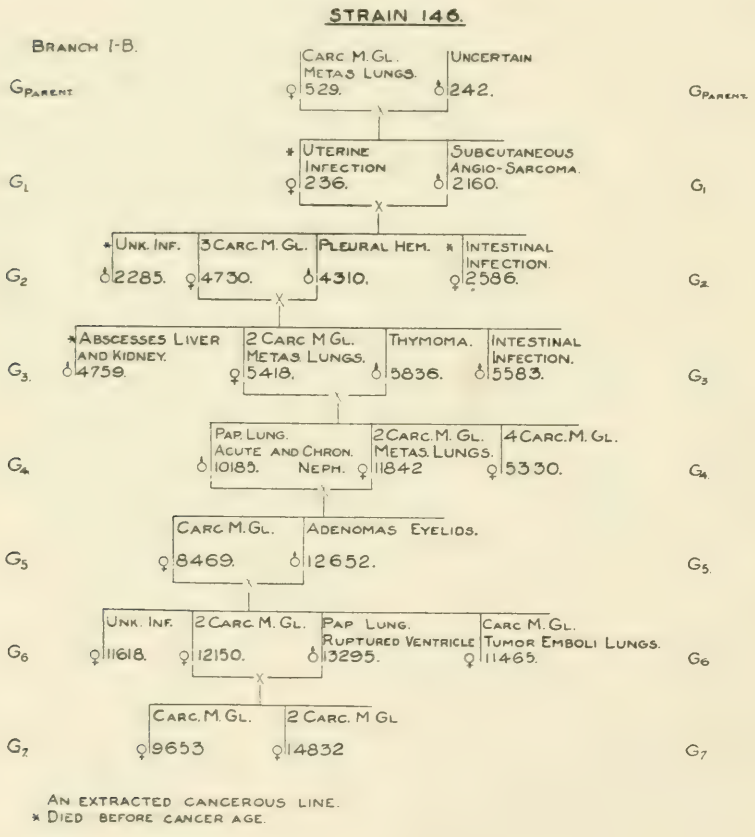


CHART 16

CHART 17

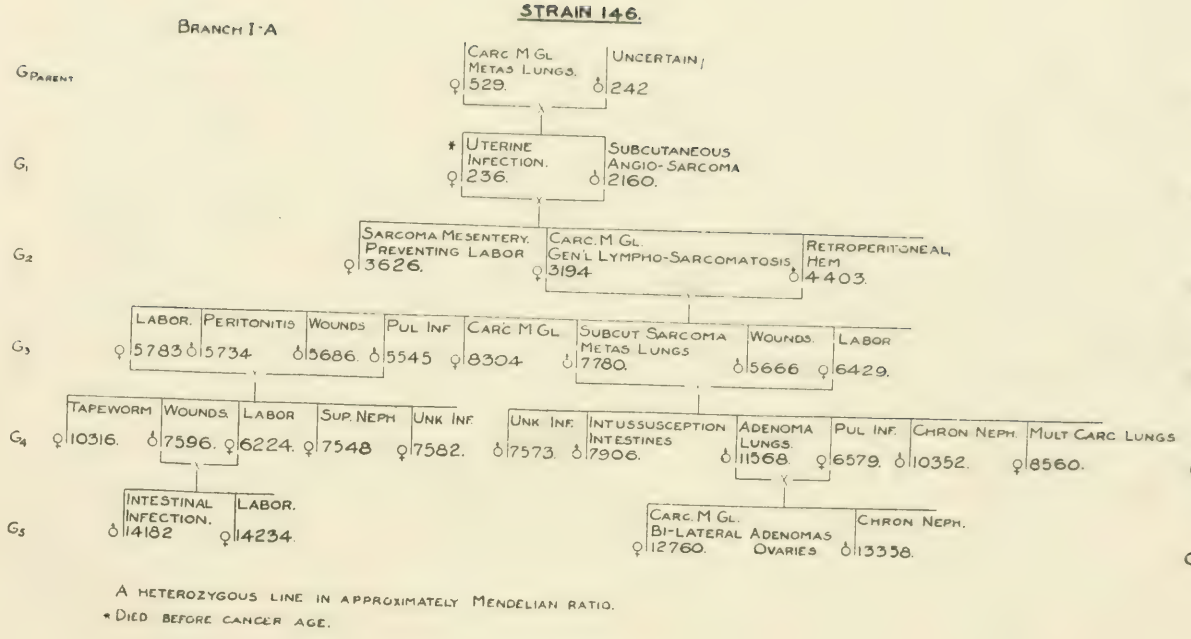
While in branch A, there arose a heterozygous line producing cancer and non-cancer in striking mendelian ratio [note also the three successive generations of sarcoma in filial generations 1, 2 and 3,] nothing remotely similar occurred with the infections represented in the strain. Throughout the three charts there is just one apparent transmission of pulmonary infection; and this is apparent only, since female 3273 was isolated with her sister, female 4886, and her daughter, female 4651, when she died of pulmonary infection. (Chart 15.) The other females in the cage caught the infection and died soon after, whereas none of her young isolated from her ever exhibited pulmonary infection. Further, the mating of female 4651, dying of pulmonary infection, with male 6144, dying of intestinal infection (both diseases being contracted after isolation from their young) failed to introduce either of these types of infection anywhere in the resulting strain, and this although it is almost impossible to keep any strain from being riddled with both these infections through contact contraction.

Note also that although the parent female of these three lines, viz., female 236, died of uterine infection, throughout all branches of the family resulting from this mating there was never another case of uterine infection. (Charts 15, 16 and 17.)

Throughout these charts, which are typical, the following facts stand out strikingly:

1. In every case where a cancerous individual is bred in, either in inbreeding or in hybridization, cancer comes out in a striking percentage in the resulting strain.

2. In no case where an individual dying of any type of well known infection is bred in, has there arisen a single case of the same infection in the resulting strain (except in one case of contact contraction) even where the families are bred out in many lines, in large numbers, and through many generations; and this although the infections tested are overwhelmingly prevalent among mice.



A HETEROZYGOUS LINE IN APPROXIMATELY MENDELIAN RATIO.
* DIED BEFORE CANCER AGE.

CHART 17

3. The cancer tendency and the non-cancer tendency segregate out conspicuously as inheritable characters which fulfill all the most exacting demands for a character which is inheritable, while there is absolutely no such segregation of any infection tendency. It is futile to *conjecture*[5] what might happen if one were dealing with "pulmonary infection" strains of mice, because one cannot get "pulmonary infection" strains of mice or any other infection strains of mice if one rules out the contact cause of transmission. On the other hand, the ruling out of contact as a cause of transmission of cancer does not modify in the least the per cent of cancer in the resulting strain.

4. From matings of mice both dying of cancer, it is possible to derive strains showing 100 per cent of cancer where the young can be kept free from infections long enough to live well into the cancer age.

5. From matings of mice where one parent dies of cancer it is possible to extract lines which follow mendelian ratios with striking precision, yielding extracted lines of cancerous, extracted lines of non-cancerous, and extracted heterozygous lines. These two facts develop consistently year after year in every test made and are not a matter of speculation. Whereas:

6. From matings of mice both dying of the same common infection (a) it is possible to derive strains which never show another case of this infection if the mice are properly protected from contact contraction, and (b) it is absolutely impossible to derive extracted strains of any common infection, if proper protection is afforded the mice from contact contraction.

7. From matings where one parent dies of a common infection, it is not possible to derive anything which approximates strains showing a segregating out of an infection tendency and a non-infection tendency, which is the hybridization test of inheritability.

8. Every mouse in the laboratory can be swept off by contact contraction of a virulent infection to which there has been exposure or, if an infected individual is properly isolated there need not

[5] Erwin F. Smith, loc. cit.

be one other case of this infection throughout the laboratory, no matter how many offspring the individual has produced.

9. Whereas, contact is absolutely ineffective and has no bearing whatever upon the inevitable appearance of cancer in strains where this tendency has been bred in.

There is no similarity whatever between the inheritance behavior of cancer and the inheritance behavior of common infections.

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COMPARATIVE PATHOLOGY OF CANCER OF THE STOMACH WITH PARTICULAR REFERENCE TO THE PRIMARY SPONTANEOUS MALIGNANT TUMORS OF THE ALIMENTARY CANAL IN MICE[1]

STUDIES ON THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

Eleventh Communication

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From the Otho S. A. Sprague Memorial Institute and the Pathological Laboratory of the University of Chicago

Received for publication May 17, 1917

GASTRIC CARCINOMA IN SPECIES OTHER THAN THE MOUSE

One of the most striking facts in the comparative pathology of cancer is the unique position of man in respect to his susceptibility to cancer of the stomach. While gastric cancer leads all other carcinomas as a cause of death in the human species, it is one of the rarest neoplasms in all other species. Thus among dogs, in which new growths are extremely common, perhaps exceeding in frequency the occurrence of neoplasms in man, cancer of the stomach is all but unknown. Fröhner (1) states that among 70,000 sick dogs there was not a single case of cancer of the stomach, although ulcers of this organ seem to occur frequently in the dog. The entire alimentary tract of all the lower animals seems to be comparatively free from cancer, if we except anal tumors in dogs (2). In his extensive review of the literature, Caspar (3) could not find a single case of cancer of the pharynx or esophagus in any species, although Cadiot (4) has described a case of carcinoma of the esophagus in a dog.

[1] Presented before the American Association for Cancer Research, New York, April 5, 1917.

The nearest thing to an exception to the statement that cancer of the stomach is extremely rare in all animals but man, is furnished by the bovines, in which squamous cell carcinoma of the rumen is by no means infrequent, for of 78 cases of cancer in bovines collected by Sticker (5) in his extensive review, 6 were found in this location. However, the rumen is properly but a dilatation of the esophagus, and not a true stomach. No case of carcinoma has ever been described in the true stomach of a bovine, so far as we can find in the literature. A similar state of affairs exists in the horse, for of 322 cases of primary carcinoma in horses, Sticker found 8 in the "stomach," but these seem to have been chiefly, if not entirely, squamous cell carcinomas of the cardia.

Sticker could find no recorded case of cancer of the stomach in sheep, swine, or cats, in all of which cancer has been observed in other locations.

Indeed, so few are the recorded cases of gastric carcinoma observed in any species of animals, beyond those of the rumen of the cow and the cardia of the horse, that they may be described in a brief space. They are so scattered through the literature, many in publications difficult of access, that it is quite impossible to hope to locate them all, but we have found the following reports, which it may be worth while to recapitulate here as a nucleus for further additions by subsequent writers.

Dog. But two apparently authentic cases of spontaneous carcinoma of the stomach have been found,[2] although in his review in 1902 Sticker collected 765 cases of primary carcinoma of other tissues in dogs. This author questions the validity of Eberlein's (6) case, but the report seems to indicate its correctness. This was a cylindrical cell carcinoma with well-developed stroma, and produced metastases in the lungs and liver. The second

[2] Hemmeter (42) says that after producing an experimental ulcer of the stomach in a dog and keeping up a high acidity, "at the autopsy a cancer of a stomach was found near the pylorus." He also describes a dog presenting an adenocarcinoma at the edge of an experimental gastric ulcer; it "reached the size of a walnut, and two metastases were found in the omentum." There are no further details concerning the character of these growths, which, if cancers, were experimentally produced rather than spontaneous.

case was observed, but not fully reported, by Magnusson (7). In discussing the occurrence of tumors of the heart in domestic animals, he mentions the case of a male dog with a carcinoma of the pylorus, which had metastasized into numerous organs, including the heart, but he gives no other details. Cases of sarcoma of the stomach have been reported in dogs (see Sticker and Caspar), but the descriptions commonly leave some doubt as to their character and origin (8).

Birds. In their review of the spontaneous tumors of birds, Joest and Ernesti (9) reported about 160 cases, of which three were cylindrical cell carcinomas of the stomach of fowls (Weiskopf, Schöppler, Zanini), as well as a much larger number in the intestine. The case of Weiskopf may be properly excluded, since there seems to be no evidence that the primary growth was in the stomach. Schöppler (10) also quoted two other cases of carcinoma of the stomach, found among 37 malignant tumors in fowls by Fujinami (11). Wernicke (12) mentioned, without giving details, still another case of carcinoma of the stomach in a chicken, described by Sticker (13). A spindle cell sarcoma of the glandular proventriculus of a male chicken, with metastases in the liver, lung, and heart, has been reported by Joest and Ernesti (9).

Bovines. In 305 cases of primary tumors of bovines observed in the slaughter house at Glasgow, Trotter (14) found no less than 25 in the rumen, of which all were squamous epitheliomas except one "scirrhous cancer;" many originated in ulcers and some in papillomata. Although there were ten malignant tumors in this series, arising in the intestines, there was no case of cancer of the true stomach. Among twenty-six cases of malignant tumors in cows, Murray (15) found 4 squamous cell carcinomas of the rumen, and the six cases collected by Sticker have been mentioned previously. We have found no case of carcinoma of the true stomach of the cow in the literature, but Magnusson (7) mentioned a case of round cell sarcoma of the "Labmagenwand" of the cow with secondaries in the heart and elsewhere, which was reported by Gänsehals (16). Caspar (3) in his review (1896), referred to several cases of "panzerartiges

Sarkom" arising in the wall of the cardia, as well as a round cell sarcoma of the stomach, but the correctness of the diagnosis of sarcomas observed so many years ago is open to question

Horses. In the eight cases of gastric carcinoma mentioned by Sticker, there is too little description to fix the origin or character of all. He described his own two cases as being respectively a "Pflaster-zell" carcinoma and a large round cell carcinoma. M'Fadyean's three cases (7), included by Sticker, were all very malignant squamous epitheliomas of the cardiac end of the stomach; one of these was inoculated into a horse without success. Apparently in the horse, as in the cow, it is the squamous cell lining that is the chief, if not the sole site of malignant degeneration. The three cases quoted by Wolff (18) are: (1) squamous cell carcinoma of the cardia, with liver metastases (Durbeck), (2) ulcerative scirrhus with metastases in the liver and peritoneum (Hillbrand); (3) squamous cell carcinoma of the cardia, at the site of ulcers produced by "Bremsenlarve" (Petit). Wolff says that sarcoma is more frequent than carcinoma, of which the commonest type is an "Ulcus carcinomatodes scirrhosum am Pansen."

Rats. Spontaneous primary tumors of the stomach of rats must be most infrequent, for among 100,000 rats examined by McCoy (19) in his plague work, in which there were found 103 tumors, there was not a single case of neoplasm anywhere in the gastrointestinal tract. The further series reported by Woolley and Wherry (20) also contained none. An interesting exception is furnished by Fibiger (21), who found in rats infected with nematodes, numerous instances of papillary carcinoma-like growths. In 19 such growths, produced by experimental infection, 4 showed minute, microscopic metastases, and 2 others seemed to be of definitely malignant structure. These were all squamous cell growths, arising from chronic inflammation in the squamous epithelial lining of the cardia of the rats, and hence more analogous to the epitheliomas of the rumen of cows or the esophageal part of the stomach of horses. Apparently this structure reacts readily by proliferation when irritated, for Bullock and Rohdenburg (22) produced polypoid

epithelial hyperplasia by introducing woolen balls soaked with chemical irritants into the stomach of rats. Adenomatous hyperplasia of the pyloric mucosa has also been observed frequently in rabbits fed lanolin for long periods by Kon (23).

In spite of the great number of albino and other domesticated rats examined in laboratories, we have found no recorded case of a spontaneous neoplasm of the stomach in these animals, and there were none among the 171 cases of spontaneous rat tumors collected or observed by Bullock and Rohdenburg (24). That this organ is at any rate, not essentially refractory to the growth of transplanted tumors, was shown by Citron (25), who obtained 25 per cent of successful inoculations of rat sarcoma in the stomach wall.

Rabbit. Neoplasms of any kind are extremely uncommon in rabbits,[3] but Schmorl (26) says:

> Twice I have found rabbits with carcinomatous tumors. In one animal it was a small carcinoma in the vicinity of the cardia, in the other a nodule in the lung with the structure of a carcinoma simplex.

We can find no further description of either of these rare cases.

Wild animals. Wild animals rarely have neoplasms of the stomach. Fox (27), however, has described a squamous cell papillary tumor arising in the pylorus of a male red kangaroo (*Macropus rufus*), with metastases in the liver, spleen, and kidneys. Renshaw (28) has reported, in the greater curvature of the stomach of a rhinoceros (about 30 years old), a triangular tumor, with the apex directed toward the pylorus, the surface flat and ulcerated; some nodules were present in the vicinity of the tumor; "the microscope showed that the tumor resembled carcinoma of man." Koch (29) reports observing a large fibromyoma ("kindskopfgross") in the stomach of a deer, arising in the rumen, and with an ulcerated, necrotic center. A fibro-

[3] Fütterer (43) describes the experimental production of gastric ulcers in rabbits, and states that in one case there occurred a glandular hyperplasia resembling an adenocarcinoma. He is careful to state that he does not claim that this is a true carcinoma, although he seems impressed with the similarity of this growth with human cancer; but neither the description nor illustrations are convincing.

myoma from the stomach of a leopard was also found by Williams (30) in the collection of the Glasgow Hunterian museum, and a similar tumor has been described in the stomach of a python by Petit and Vaillant (31), which is the only case of stomach tumor of any kind in a cold blooded animal that we can find, except the very incomplete information contained in a statement made by Kantorowicz (32) who, in a discussion of another case of cancer, said that he had seen a pyloric carcinoma, with multiple liver metastases, in a three year old "Box."[4] Williams (2) quotes the case, reported by Harrison, of a male lion, sixteen and a half years old, with a columnar cell carcinoma of the ileum above the ileocecal valve. Fox has also described an adenocarcinoma of the intestine with many metastases in a male dasyure.

Of particular interest, in view of the rarity of tumors in primates, is the report by the same author (33) of a diffuse adenoma of the stomach in a male Hamadryas baboon, the only neoplasm found by him in autopsies on 381 primates. A definite case of primary carcinoma of the stomach in a primate, however, has been reported by Schmey (34), occurring in a female long tailed monkey (*Cercopithecus rufo viridis*), aged eight to ten years. It involved the stomach so diffusely that the point of origin could not be determined, infiltrated through to the serosa in many places, and produced extensive lymph node metastases. The tumor was a small, polymorphous cell medullary carcinoma, of the solid type (without tubule formation), and did not differ in any essential particular from human gastric cancer.

GASTRIC CARCINOMA AND SARCOMA IN THE MOUSE

In spite of the enormous number of mice autopsied in laboratories, and the frequency of cancer in these animals, we can find but four reported cases of primary neoplasm in the stomach of this species. These cases are as follows:

1. Murray (35) has reported a case, which is so typical of all the others, that we quote his description in full:

[4] Presumably this is the teleostean fish, *Box vulgaris* (Cuvier).

In performing the autopsy of an old male mouse the spleen was seen to be adherent to the greater curvature of the stomach. On opening the stomach along the lesser curvature, a small ulcer was discovered with thickened edges at the point of adhesion to the spleen. The normal mucous membrane of the stomach in the mouse consists of two parts sharply marked off from each other. The cardiac two-thirds is lined by stratified squamous epithelium directly continuous with that of the oesophagus, and comparable to the rumen of herbivora. The pyloric portion, much smaller in extent, forming one-third of the whole organ, is lined by a glandular epithelium similar to that of other mammals. The ulcer under discussion is situated in the squamous-celled portion immediately adjacent to the glandular part. The squamous-celled alveoli extend laterally under the adjacent glandular and normal squamous mucous membranes. They have infiltrated and expanded the circular muscular coat, extended through the longitudinal layer, and expand under the peritoneal covering into a small cyst. Isolated alveoli lie external to the deeper layer of this flattened cyst between the adjoining lobes of liver and pancreas.

2. In concluding the above report Murray merely mentioned a second case, as follows: "An earlier stage of the same condition was discovered at autopsy in an aged female mouse, which presented a carcinoma of the right axillary mamma." He published a photograph of the gross appearance of the stomach, and merely added that it resembled closely the more advanced case.

3. Little and Tyzzer (36), in a paper on the "Inheritance of Susceptibility to a Transplantable Tumor," described a case as follows:

A carcinoma of the stomach, which originated in a laboratory stock of wild mice, grew when implanted in four of eleven, but on the second transfer failed to grow in twelve mice of the same stock.

In a letter Dr. Tyzzer has given us further information concerning this case, which he kindly permits us to publish. He states that it was in a female wild mouse, and adds:

The mouse in which the original tumor occurred was inoculated with a tumor about a year and seven months previously. This apparently

failed to develop, as subsequent negative observations indicated. It was killed on account of the appearance of a rounded tumor beneath the skin. This had the general appearance of a sebaceous cyst except for a mass of pinkish tissue in one portion of the wall. Axillary and inguinal lymph nodes were not enlarged. No metastases were apparent in the lungs. There was a mass of tissue infiltrating the greater curvature of the stomach, measuring 3 mm. in thickness and somewhat over 1 cm. in extent. The left ovary was replaced by a mass of similar tissue. There were nodules distributed along the uterus and numerous small masses having the distribution of the post-peritoneal lymph nodes. These latter metastatic nodules were used for the transplantations. The transplants appeared as rounded nodules but on section were found to contain a collection of fluid which turned litmus paper blue.

The histological examination of the subcutaneous nodule showed a tumor the epithelium of which corresponded with that of other mammary tumors of the mouse. There was, however, a tendency of the epithelium to undergo a sort of pseudo-cornification; that is, there were foci and layers of swollen red-staining cells. The tumor of the stomach was composed of epithelium having a distinct epidermoid tendency in its differentiation. The squamous epithelium of the cardiac portion of the stomach showed a very marked papillary change and the tumor epithelium resembled this so closely that it appeared to me most probable that it had arisen in this portion of the stomach. The tumor showed marked invasive and metastasizing qualities so that it had replaced the muscular layers of the stomach and it was found widely distributed throughout the lymph nodes of the abdominal cavity.

4. Itami (37) has reported a case from the Crocker Fund laboratory, occurring in a white female of unknown age, from which two adenocarcinomas had been extirpated, without recurrence, about twelve weeks before it was killed because of progressive loss of weight. The squamous portion of the stomach was diffusely thickened by a squamous cell carcinoma, which did not involve the pyloric portion. There were no metastases. Forty-eight mice were inoculated from the cancer, without any takes.

To these four cases we wish to add four more, which represent all the instances found in 16,500 autopsies on mice, nearly

all dying a natural death, all without experimental manipulation or interference with their natural development, mostly at advanced age, and in a stock which has yielded approximately 2000 mice with tumors of other sorts. Every suspicious spot in any organ has been examined microscopically, and many inflammatory lesions of the stomach thus excluded.

5. A male mouse (no. 5802), two years old. Near the pyloric portion of the stomach is a mass about 15 by 15 by 12 mm., occupying the wall of the stomach, and growing out towards the pancreas, which it does not seem to invade. The external surface of the stomach is marked out by irregular nodules. The internal surface is ulcerated, folded, slightly papillomatous, and somewhat discolored in the cardiac portion, both anterior and posterior surfaces being involved, but not the pylorus. The wall varies from 3 to 5 mm. in thickness.

In the mesentery is a roughly spherical white mass about 11 or 12 mm. in diameter, not adherent or infiltrating. The central portion is softened, and the periphery is nodular. No nodules are found elsewhere. The spleen and liver are large and full of amyloid; the kidneys show but little change; the lungs exhibit no gross changes and especially no metastases.

Microscopically, the primary tumor is seen to be composed of masses of squamous epithelium, forming typical cancer nests with hornified centers (fig. 1). The growth extends to the junction of the squamous and cylindrical epithelial portion of the stomach, and spreads freely from the former under the latter, which is not ulcerated. For the most part, the cancer tissue is necrotic in the center, but is not much ulcerated except at one point where there is ulceration through to the serosa. The cancer replaces all the coats of the stomach, and protrudes into granulation tissue and adhesions attached to the serosa. The pancreas is firmly adherent to the stomach over the tumor, and there is a slight invasion of the superficial portion of the pancreas. Mitotic figures are abundant in the peripheral parts of the growth.

The mesenteric nodule consists of a central necrotic mass, containing many large bacteria without cellular reaction (post

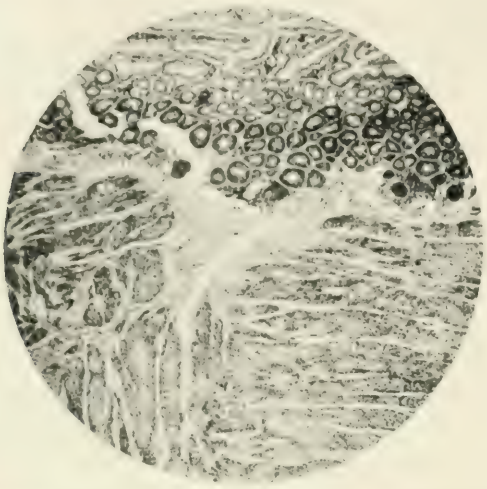

FIG. 1. Photograph showing the typical squamous cell growth infiltrating beneath the glandular mucosa of the pyloric portion of the stomach. × 70. (Case 5, our no. 5802.)

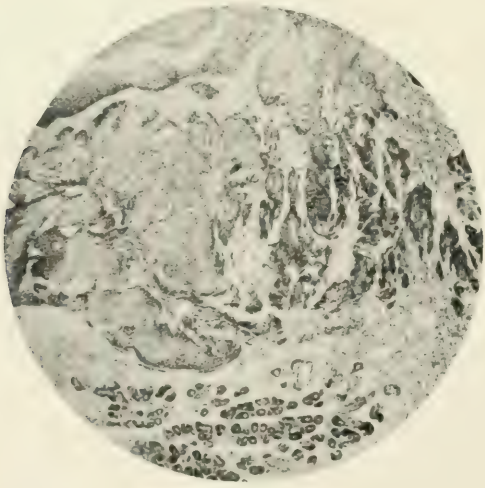

FIG. 2. Showing the growth of cancer as epithelium from the squamous cell lining of the stomach, through the muscularis, into adhesions binding the pancreas to the stomach. × 60. (Case 6, our no. 7851.)

mortem invasion), with a narrow peripheral zone of plugs of squamous cells. There is a relatively thick capsule of young connective tissue infiltrated with round cells and, in some places, with collections of polymorphonuclear leucocytes; it is invaded freely by the cancer cells, which show very few mitotic figures. No recognizable lymph node tissue is found. The spleen contains much amyloid, the liver less, and the kidneys very little; otherwise these organs show no noteworthy changes. The lungs are normal and no tumor emboli are found.

Diagnosis. Squamous cell carcinoma of the cardiac portion of the stomach, with metastasis into mesentery. General amyloidosis.

6. This was also a male (no. 7851), twenty-five months old, and the uncle of mouse 5802. The cardiac end of the stomach is diffusely thickened and indurated with coarse lobulations visible externally, its internal surface being slightly ulcerated and thrown into small folds. The ulcer measures 16 by 12 mm. and is 8 mm. thick at the thickest part. The rest of the stomach is full of pus. No parasites are found in the stomach. The liver is small, hyperemic, and adherent to the stomach. The spleen is stiff with amyloid but not much enlarged; the kidneys are small and hard; the lungs bloody, but without tumor nodules. No metastasis could be found.

Microscopically, the growth is limited to the cardiac portion of the stomach, where the entire squamous epithelial surface is underlaid by masses of typical squamous cell carcinoma nests with extensive hornification. The growth has almost completely replaced the muscular layers and infiltrated the serosa (fig. 2). The adhesions between the liver and stomach are infiltrated with cancer nests, but the liver itself is not invaded. Mitotic figures are scanty. In one place, a large pus sac is found, surrounded by a layer of stratified epithelium on a granulation tissue base. The spleen and liver show advanced amyloidosis, and the lungs are edematous. No metastases are found in any place.

Diagnosis. Squamous cell carcinoma of the cardiac portion of the stomach. General amyloidosis.

7. Since the preceding two cases were reported to the Association for Cancer Research, we have obtained a third cancer of the stomach quite similar to them. This occurred in a female mouse, no. 16440, aged about 27 months. The animal was second cousin to female no. 12614, reported in this paper.

The stomach is greatly enlarged, measuring about 25 by 15 by 10 mm. At the junction of the cardiac and pyloric portions of the stomach is a thickened mass of tissue, extending nearly around the stomach, only about 3 mm. of the dorsal side being of normal thickness. This thickened portion at its widest point on the ventral side of the stomach is about 15 mm.; on the dorsal side it is 3 to 5 mm. This thickening does not extend to the pylorus, the pylorus and the pyloric portion of the stomach being quite normal in appearance and thickness for a distance of 3 to 5 mm. The surface of the tumor is slightly hemorrhagic, and although somewhat lobulated it is smoother than the other tumors studied. The consistence is quite firm and the thickness is uniformly 2 to 3 mm. The inner surface appears only slightly ulcerated. On the ventral aspect there is a hard white nodule, 3 mm. in diameter, in the omentum. There is a similar larger white nodule in the root of the mesentery, which is about 5 mm. in diameter.

There was nothing noteworthy elsewhere. The lymph nodes were very slightly enlarged; the spleen was much enlarged. There was no indication of metastases elsewhere.

Diagnosis: Carcinoma of stomach with metastases in regional nodes.

Microscopically, both the squamous and glandular surfaces are underlaid by a most diffuse and extensive squamous cell growth, involving all structures from the serosa to the lumen, and almost completely replacing the muscularis. Cancer nests are occasionally found, also, among the tubules of the mucosa, but there is here very little ulceration. There is more ulceration in the squamous portion of the stomach. Hornification is often seen, but is not prominent; mitotic figures are rare.

The nodule in the omentum consists of a solid mass of cancer tissue without evidence of any pre-existing lymphatic tissue.

Here the growth consists of a solid mass of densely packed plugs with much hornification. There is a similar microscopic nodule adjacent to the larger one.

The lymph node at the root of the mesentery shows a nodule similar to that in the omentum, although with more hornification; some of the lymphatic tissue still remains.

The liver and spleen show extensive amyloid deposit, especially the former. No metastatic growths are found in either of these organs, or in the lungs.

It will be noted that all these seven cases involve the cardiac portion of the stomach, and are squamous cell cancers. This brings them into the same category with the stomach cancer of the cow and horse, which involve almost or quite exclusively the squamous portion or rumen. Especial interest, therefore, attaches to the following case, which concerns a cancer arising from the glandular, secretory pyloric portion of the stomach.

8. A male mouse, aged two years and seven months (no. 14580). In the lower part of the pyloric portion of the stomach the wall is greatly thickened, practically occluding the lumen, and forming a solid mass about 8 mm. in diameter. The wall of the stomach here is 1.5 to 2 mm. thick, the external surface being smooth. This is not adherent to the adjacent structures, except the pancreas, and is covered by a smooth serosa. A few of the adjacent peripancreatic lymph nodes are slightly enlarged. In the stomach is a hair ball, and the pyloric mucosa is thrown up into longitudinal folds and superficially ulcerated. The lower part of the intestines contains a bloody fluid. The liver is pale and slightly enlarged, but not involved in the tumor. The spleen is enlarged to about four times its normal size. There are no secondary nodules to be found in any other viscera, and these show no marked changes, except that the seminal vesicles are atrophied and the left cardiac ventricle is hypertrophied.

Microscopically (fig. 3), the growth involves exclusively the glandular, pyloric portion of the stomach. Passing insensibly from the uninvolved portion of the mucosa, which shows much evidence of chronic inflammatory changes, is a growth of atypical

tubular structure which infiltrates freely through the muscularis, and even invades the adherent pancreas. Very few mitotic nuclei are seen. The tubules are generally distended and exhibit a low, flattened epithelial lining, much resembling a "colloid cancer" of the stomach, except that the lumina of the tubules contain no mucin or other stainable material. There is a moderate round cell infiltration in the growth, and considerable stroma formation, with much fibrous thickening in the serosa

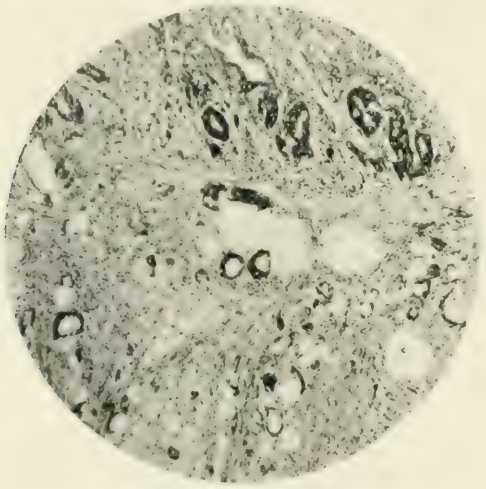

FIG. 3. Tubular carcinoma of pyloras infiltrating the submucous and muscular layers, with dilatation and pressure atrophy in many of the tubules. The glandular mucosa is seen in the upper part of the section, exhibiting round cell infiltration and fibrosis, presumably resulting from the presence of a hair ball. × 90. (Case 8, our no. 14580.)

where it is adherent to the pancreas. This fibrous tissue is extensively infiltrated by tumor tissue with collapsed tubules, and simple cords of cells.

This growth resembles very closely in its appearance the tubular carcinomas of the stomach seen in man.

Section of two of the regional lymph nodes shows them to contain numerous tubules, some isolated and others in clusters, with a little fibrosis about the infiltrating tumor tissue.

No metastases are found in the lung or liver.

Diagnosis. Primary tubular carcinoma of the stomach, with metastasis into regional lymph nodes. Hair ball in the stomach, with chronic gastritis.

Recapitulation. Of the eight cases of carcinoma of the stomach of mice now recorded, seven were squamous cell carcinomas involving solely the cardiac portion, and only one corresponds to the tubular, cylindrical cell type of cancer found in man. Four occurred in females, and four (including three of our four) were in males. Four showed metastases into the regional lymph nodes, one having in addition an ovarian metastasis. Two also had primary mammary gland carcinoma. Inoculation was tried with only two, and in one of these a few takes were obtained in the first generation, which could not be carried to a second generation.

We have observed no instance of secondary carcinomatous growths in the stomach among approximately 2000 mice with spontaneous tumors.

We have found no recorded case of sarcoma involving the stomach in the mouse, although sarcoma is by no means a rare growth. Thus, in a recent report (38), we collected from the literature 17 cases of sarcoma in mice, and recorded in addition 87 cases observed in 12,000 autopsies on the Slye stock, but none of these sarcomas arose in the stomach. Since then we have observed a case that resembles very closely a primary sarcoma of the stomach. The record is as follows:

Female (no. 12614), age twenty months. Beginning at the duodenum, and spreading up into the cardiac portion of the stomach, is a diffuse growth of tissue forming a mass about 20 by 18 mm. in diameter, and up to 12.5 mm. thick. It is pink and fleshy, covered by intact serosa, and without exudate or necrosis on either serous or mucous surface. The external surface is coarsely lobulated and the growth is mostly posterior, leaving the anterior surface of the stomach relatively free. This infiltration also surrounds the common bile duct and follows it up into the hilum of the liver. In the bile duct is a tape worm, but the typical extensive suppurative cholangitis and hepatitis commonly found in these cases, is not present. The liver does,

however, contain several small nodules of tissue resembling that of the tumor. The extreme cardiac end of the stomach is shrivelled and empty. A mass in the mesentery, fitting over the stomach like a cap, but not adherent to it, measures 20 by 12 mm. across and 8 to 10 mm. thick. From this a few strands run to a smaller mass in the mesentery. The diaphragm is thickened and on the right side shows a mass of neoplastic tissue. No nodules were found in the lungs.

The spleen, ovaries, and the lymph nodes in general, were not noticeably enlarged.

Microscopic appearances. Infiltrating the stomach wall diffusely, and widely separating the mucosa from the serosa, is a uniform growth of small, nearly spherical cells (figs. 4 and 5). These are somewhat larger than lymphocytes, less spherical, and have generally much less intensely staining nuclei. They are not uniform in size, although there are no very large cells and no multinucleated forms The cytoplasm is scanty, and stains rather deeply, resembling somewhat the cytoplasm of plasma cells. The nuclei vary from those with chromatin as dense as that of a lymphocyte, to vesicular and occasionally lobulated forms; generally the chromatin occurs in closely packed fine granules. No mitotic nuclei are seen, but there is frequent amitotic division. The growth is entirely uniform throughout, and infiltrates and destroys the muscular tissue freely, so that in most of its extent no muscle cells remain. There is no inflammatory reaction of any kind, and the mucosa is everywhere intact. The serosa is infiltrated, without any adhesion or fibrous proliferation, but there is very little invasion of the mucosa. There are a few blood-vessels, but there is no necrosis or other retrogressive change. Mitoses are not found. The growth involves both the pyloric and cardiac portions of the stomach.

A similar tissue is found infiltrating the hilum of the liver along the bile ducts, and in places distinct nodules have formed in the liver. There is some suppuration in the larger bile ducts. Beyond invasion by the tumor, the liver shows little change, and does not exhibit the perivascular infiltration characteristic of leukemia and pseudoleukemia.

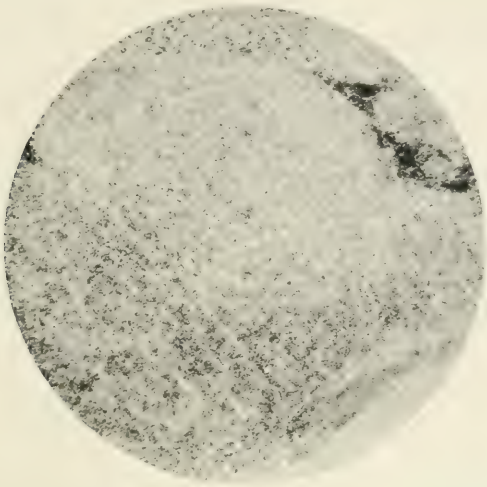

FIG. 4. Very low power view to indicate the extent of infiltration by sarcoma tissue of the stomach wall, which is shown in the entire thickness in one of the thinnest parts of the invaded wall. Above, the glandular mucosa can be seen, and the dark patches of lymphoid cells serve to indicate the relative size and nuclear density of the neoplastic tissue. × 45. (Our no. 12614.)

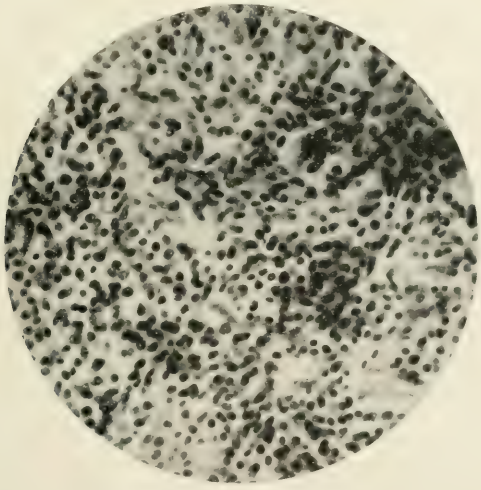

FIG. 5. High power view of the tissue in the stomach wall, reproduced in figure 4, to show the cell structure. The entire neoplastic tissue in this case is of similar structure. × 450.

The kidneys and lungs likewise show no evidence of lymphoid deposits, although the latter does contain a few small nodules of tissue, apparently metastatic growths of hematogenous origin, resembling, in location and character, typical tumor metastases rather than leucocytic infiltration or lymphoid hyperplasia.

The spleen contains much amyloid, and shows also irregular patches of tissue similar to that in the stomach and liver; this contrasts sharply with the lymphoid tissue of the spleen, the cells being paler, slightly larger, and with more cytoplasm. There are no definite nodules of neoplastic tissue in the spleen.

The diaphragm is infiltrated with the same type of tissue, which destroys the muscle, and in one place forms a distinct nodule.

A huge mass of mesenteric lymph nodes, is present. Two of these, measuring respectively 7 and 9 mm. in diameter, are so infiltrated with tissue of the same character that almost no lymphoid tissue can be found.

The blood-vessels show no excess of leucocytes in the circulating blood.

We are by no means sure of the correct diagnosis in this case. In nearly all respects it corresponds to a small round cell sarcoma, arising in the wall of the stomach and producing the type of local extension usual in lymphosarcomas; very similar lymphosarcomas, furthermore, have been described in the stomach and intestine of man. The only grounds for reluctance in diagnosing this as a primary lymphosarcoma of the stomach are, first, the general doubt as to the real character of all growths of this lymphosarcomatous character, especially emphasized by their unsolved relation to the pseudoleukemias. Second, we find in the spleen a diffuse growth of cells similar to those of the tumor, which do not occur in distinct nodules as typical secondary tumors should, but rather resemble the results of a hyperplastic change in spleen cells. We have seen so many instances of tumor-like proliferations in the liver, associated with diffuse growth of similar cells in the spleen, and apparently presenting in different cases various stages between conditions that seem almost certainly inflammatory or granulomatous, to

others not certainly distinguishable from true sarcoma, that we have become very doubtful of the advisability of making positive diagnoses of sarcoma in related conditions. They sometimes recall certain of the cases of Gaucher's disease and other splenomegalic conditions. Nevertheless, in most respects this case seems to be as typical a sarcoma as any gastrointestinal lymphosarcoma described in man, and we are quite unprepared to say that it is not a sarcoma. The gross findings are even more characteristic of sarcoma than the microscopic, but it is impossible to say whether the growth arose primarily in the stomach or in the mesenteric lymph nodes.

INTESTINAL CARCINOMA IN THE MOUSE

Although intestinal cancer is not extremely rare in the lower animals, especially in birds, yet in mice it is no more common than cancer of the stomach. In the literature we have found mention made of this condition only by Murray. In the Third Scientific Report of the Imperial Cancer Research Fund (p. 71), he says that carcinoma of the intestine has been reported by Bashford, Murray, and Cramer, and by Twort. We have found no case of adenocarcinoma, similar to those described from the London laboratories, in our mice. The only intestinal carcinoma in the Slye stock occurred under the following conditions:

A male (no. 8345) twenty-seven months old, a cousin of mouse 7851, which had cancer of the stomach, developed a prolapse of the rectum and was kept under observation in the "hospital" for the six months preceding his death. About four months before death, it was noticed that the prolapsed portion of the rectum had become ulcerated. The hair became scanty, and the mouse emaciated greatly. The protruded rectum became much indurated, and somewhat ulcerated, forming a hard mass about 6 mm. in diameter at the time of death. Autopsy disclosed no other noticeable abnormalities, beyond the reduced size of the organs associated with the emaciation.

Microscopically, the mucosa lining the prolapsed part of the bowel is practically normal, but that covering the protruded portion is much altered. The mucosa and submucosa show extensive chronic inflammatory changes, and the mucous glands are much hypertrophied, many of them also being dilated to cystic proportions. Part of the surface is covered by squamous epithelium which overlies mucous glands, and, being discontinuous, is apparently of metaplastic origin, at least in part. Some

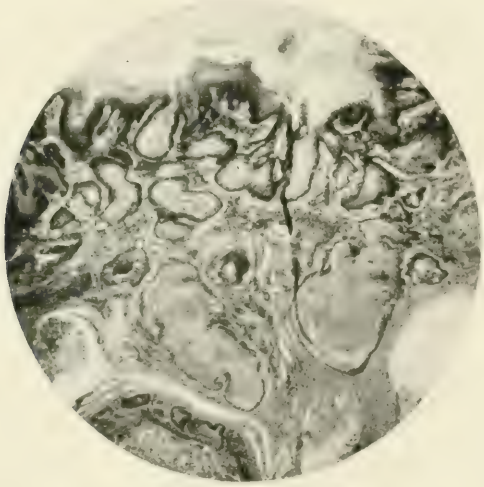

FIG. 6. Section through prolapsed rectum, showing the heavy squamous epithelial coat that covers the external surface of the bowel. From this the squamous cell plugs infiltrate through the coats of the bowel to the tubular glands of the rectum, seen in the lower left hand corner. × 60. (Our no. 8345.)

areas of this squamous epithelial coat show hyperplasia with the development of downgrowths of columns of epithelium, and in one considerable area the downgrowth has assumed a distinctly malignant character (fig. 6). Here the columns of squamous epithelium spread extensively into the muscularis, and grow freely in the lymphatic channels, in places penetrating nearly to the mucosa of the internal portion of the rectum. The centers of the epithelial plugs show marked hornification, and outside them is a moderate round cell proliferation. The

regional lymph nodes show no metastasis. There are no changes of interest in the other tissues.

Diagnosis. Early squamous cell carcinoma developing in the mucosa of a chronically inflamed, prolapsed rectum.

DISCUSSION

The demonstrated fact that the alimentary tract of all the lower animals is so rarely the seat of primary tumors, affords such a striking contrast to the frequency of malignant change in the stomach and intestines of man, that it would seem probable that herein must lie important evidence as to the etiology of cancer. It is evident that in the gastric cancer of animals, as elsewhere, chronic injury is important; witness the prevailing location of the disease in the cardiac portion or rumen, where the hard food materials are held, and where simple ulceration is common. The single case of carcinoma of the pylorus in a mouse apparently resulted from the irritation of a hair ball. Fibiger's neoplasms in the rat stomach are evidently the result of irritation rather than of specific infection. It is of interest that despite the prevalence of macroparasites in the alimentary canal of all the lower animals, neoplasms of this tract are so infrequent. We note also that in none of the eight recorded cases of cancer of the stomach in mice was there any evidence of the presence of animal parasites, and hence they are not analogous to Fibiger's observations.

The frequency of gastric carcinoma in man, naturally, is commonly attributed to the special character of his food, which differs from that of all other animals solely in that it is cooked and seasoned. Its actual materials can scarcely be held responsible, since carnivora, herbivora, and omnivora are equally free from gastric cancer. The possibility that the heat of our foods may be responsible has been an attractive hypothesis, and the evidence on this subject has been so well and so recently reviewed by Lerche (39), that further discussion is not required here. This author found the evidence very convincing that carcinoma of the stomach and esophagus is related to, and

probably dependent upon, the use of hot foods and drinks. He calls particular attention to the correlation of the well-known variation in geographical distribution of cancer of the stomach with the practice of the populations in respect to hot food and drinks, for cancer of the stomach is frequent in the north temperate zone where much hot tea and coffee are consumed, and rare among tropical and sub-tropical peoples who do not use these beverages. The chief objection to this hypothesis lies in the fact that carcinoma arises most often in the pyloric region, whereas it would be expected that hot food and drink would most affect the esophageal orifice and the cardia. Lerche collects much evidence that corrosive fluids produce their chief effects along the lesser curvature and in the pyloric region, particularly the latter. On the other hand, the direct measurements of the temperature of the stomach contents by Stengel and Hopkins (41) indicate that the fundus is much more affected by changes in the temperature of the food than is the pylorus. It would seem probable, on the basis of all these observations, that the food first impinges on the lesser curvature and the pylorus, to come to rest in the fundus; if this be correct, the influence of the hot food as an irritant might be greater at the pylorus and lesser curvature than at the cardia, although the average temperature might be more modified by food in the cardia. Whenever hot foods or drinks are taken, the greater curvature and cardia would be protected by the materials already present, unless the stomach were absolutely empty, whereas the mucosa of the lesser curvature and pylorus would come in momentary contact with the hot foods undiluted.

The influence of condiments in foods might also well be of importance, in view of the profoundly irritating character of most of them, but unfortunately we have no reliable statistics as to the frequency of cancer of the stomach in such countries as Mexico, where fiery condiments are most used. However, Williams (2) quotes Jourdanet as stating that cancer is almost unknown in the hot regions of Mexico, whereas in the cool regions on the high plateaux it is frequent. This statement does not differentiate between cancer of the stomach and the other

locations, unfortunately. The figures on cancer mortality in Mexico City, quoted by Hoffman (40), indicate a low cancer rate without any particular frequency of gastric cancer, but the reliability of these data is open to question.

Another possible factor, which we have not seen mentioned, is the chemical change in food that is produced in cooking. It is generally recognized that the products of incomplete combustion of organic materials have a particularly marked tendency to irritate and stimulate the skin to the point of malignant degeneration, as seen in chimney sweeps, and in paraffin, pitch, and tar workers. May it not be possible that in the cooking of foods similar products are formed, especially where the heat is high, as on the surface of baked and roasted foods? Domesticated animals, although fed on cooked foods to some extent, probably never have them constitute so large a proportion of their diet as man.

It would seem impossible to escape the conclusion that there is something about the preparation, composition, or manner of preparing food that accounts for the frequency of cancer of the stomach in man, for surely mere mechanical irritation, and acute ulceration, are at least equally conspicuous in the stomachs of the lower animals. This striking difference affords a clue for profitable investigation as to the cause of gastric cancer.

SUMMARY

A review of the literature shows that in all animals except man, carcinoma of the stomach is extremely uncommon and the recorded cases found in the lower animals are here collected and discussed briefly. In animals with a rumen, or in which the cardia is lined by squamous epithelium, carcinoma o this tissue is observed much more frequently than in the glandular gastric mucosa. Only four cases of carcinoma of the stomach of mice could be found recorded in the literature, all in the squamous cardiac portion. In 16,500 autopsies on mice dying natural deaths at all ages in this laboratory, we have found three squamous cell carcinomas of the cardia, and one tubular pyloric

carcinoma; the latter apparently resulted from the presence of a hair ball in the stomach. One gastric neoplasm, resembling closely a sarcoma, was also observed. Carcinoma of the intestine is also very rare in mice, the only case observed in the Slye stock being a squamous cell carcinoma arising in the external surface of a chronically prolapsed rectum. The significance of man's susceptibility to carcinoma of the stomach is not yet known, but probably it depends on the heat of his food or the condiments employed in seasoning it or on the chemical changes produced by cooking.

REFERENCES

(1) Fröhner: Cited by Wolff (18).
(2) Williams: Natural History of Cancer, 1908, p. 89.
(3) Caspar: Ergebnisse allg. Path., 1896, iii, 754.
(4) Cadiot: Cited by Sticker (5).
(5) Sticker: Arch. f. klin. Chir., 1902, lxv, 616 and 1023.
(6) Eberlein: Monatsh. f. prakt. Thierheilk., 1897, viii, 289.
(7) Magnusson: Ztschr. f. Krebsforsch., 1915, xv, 212.
(8) Besnoit: Revue vétérin., 1895, p. 486.
(9) Joest and Ernesti: Ztschr. f. Krebsforsch., 1915, xv, 1.
(10) Schöppler: Ztschr. f. Krebsforsch., 1913, xiii, 332.
(11) Fujinami: Gan, Tokio, 1908, ii, 61.
(12) Wernicke: Ztschr. f. Krebsforsch., 1911, x, 168.
(13) Sticker: Der Krebs der Vögel, Geflügelbörse, 1907, No. 43.
(14) Trotter: Jour. Comp. Path. and Therap., 1911, xxiv, 1.
(15) Murray: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, p. 41.
(16) Gänsehals: Ztschr. f. Fleisch. u. Milchhyg., 1908, xviii, 355.
(17) M'Fadyean: Jour. Comp. Path. and Therap., 1899, xii, 139; Jour. Comp. Path. and Therap., 1900, xiii, 339.
(18) Wolff: Die Lehre v. d. Krebskr., Teil III, 1913, 233.
(19) McCoy: Jour. Med. Research, 1909, xvi, 285.
(20) Woolley and Wherry: Jour. Med. Research, 1911, xxv, 205.
(21) Fibiger: Ztschr. f. Krebsforsch., 1914, xiv, 295.
(22) Bullock and Rohdenburg: Proc. Soc. Exper. Biol. and Med., 1915, xii, 161.
(23) Kon: Verhandl. japan. path. Gesellsch., 1916, vi, 178.
(24) Bullock and Rohdenburg: Jour. Cancer Research, 1917, ii, 39.
(25) Citron: Centralbl. f. Bakteriol., 1913, lxxii, 328.
(26) Schmorl: Verhandl. d. deutsch. path. Gesellsch., 1903, vi, 136.
(27) Fox: Jour. Path. and Bacteriol., 1912, xvii, 217.
(28) Renshaw: Abstract in Ztschr. f. Krebsforsch., 1904, i, 347, without reference to the original article, which we have not been able to find.

(29) Koch: Verhandl. d. deutsch. path. Gesellsch., 1904, vii, 136.
(30) Williams: Natural History of Cancer, 1908, p. 110.
(31) Petit and Vaillant: Bull. Mus. Nat. Hist., Paris, 1902.
(32) Kantorowicz: Berl. klin. Wchnschr., 1914, li, 1435.
(33) Fox: Forty-second Report, Philadelphia Zool. Soc., 1914.
(34) Schmey: Berl. klin. Wchnschr., 1914, li, 1435.
(35) Murray: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, p. 71.
(36) Little and Tyzzer: Jour. Med. Research., 1916, xxxiii, 396.
(37) Itami: Proc. New York Path. Soc., 1916, xvi, 170.
(38) Slye, Holmes and Wells: Jour. Cancer Research, 1917, ii, 1.
(39) Lerche: Surg., Gynec., and Obst., 1916, xxiii, 42.
(40) Hoffman: Mortality from Cancer throughout the World, Newark, 1915, p. 758.
(41) Stengel and Hopkins: Am. Jour. Med. Sc., 1917, cliii, 101.
(42) Hemmeter: Am. Jour. Med. Sc., 1903, cxxv, 676.
(43) Fütterer: Ueber die Aetiologie des Carcinoms, 1901.

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THE EFFECT OF ALCOHOL ON THE REPRODUCTIVE TISSUES.

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PLATES 57 TO 60.

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That alcohol may produce distinct anatomical changes in the testicles of those who use it in excess has been observed from time to time by pathologists for many years, and yet this fact does not seem to be widely known or generally taken into account in considering the effects of alcoholism.

HISTORICAL.

As long ago as 1837, Rösch[1] described degenerative changes in the testicles of alcoholics, and Lancereaux described parenchymatous degeneration of the seminal tubules. In 1877 Schlemmer[2] discussed the effects of alcoholism on the testicles, and noted that it often caused fatty degeneration of the seminal epithelium, decrease in the number of spermatozoa, and pigmentation of the seminal fluid. Rheinstaedter[3] (1879) and Curschmann[4] attributed loss or decrease in testicle function to chronic alcoholism, the former stating that in his experience excessive beer drinking had a greater effect than wines or distilled liquors.

Busch[5] (1882) studied the testicles in 100 consecutive autopsies of adult males and found 27 without spermatozoa, 39 with few, and 34 with many. Although phthisis and other chronic diseases furnished many cases of azoospermia, he observed that this condition occurred frequently in alcoholics, especially when associated with cirrhosis.

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[1] Rösch, cited by Forel.[13]

[2] Schlemmer, A., *Vrtljschr. gerichtl. Med.*, 1877, xxvii, 444.

[3] Rheinstaedter, cited by Busch.[5]

[4] Curschmann, cited by Busch.[5]

[5] Busch, A., *Z. Biol.*, 1882, xviii, 496.

In 1898 Simmonds[6] reported the study of 1,000 male bodies, of which 125 showed azoospermia. As previously observed by Busch, Simmonds found chronic diseases responsible for many cases, the incidence of azoospermia in cancer cases being 7 per cent, in chronic nervous diseases 14 per cent, in phthisis 18 per cent, while of the chronic alcoholics 60 per cent had no spermatozoa. Of all autopsied males, 5 per cent were sterile through the use of alcohol, as against 3.3 per cent from genital diseases. He also found that mechanical occlusion of the seminal tract, even of years' duration, seldom causes atrophy of the testicles or absence of spermatozoa in the tubules.

Bertholet[7] (1909) made a report of the findings in 75 bodies of men dying at from 15 to 91 years of age, of whom 39 were habitual drinkers. Of the 39 alcoholics, only 2 had testicles that did not show atrophy of the parenchyma and sclerosis of the stroma. The most marked change occurred in alcoholics who died of tuberculosis or cirrhosis. Of the two alcoholics without atrophy, one was a man but 24 years old, and the other died of an acute intestinal obstruction. Bertholet describes the changes in detail. Generally the testicles are noticeably small and firm, because of atrophy of the epithelium and increase in the fibrous tissue. The spermatogonia are highly atrophic, the nuclei cannot be stained distinctly with hemalum, and it is impossible to distinguish between spermatocytes and spermatids, because they are all in the resting stage and show no mitosis or spermatozoa production. The cells of Leydig, on the other hand, seem to be increased in size, number, and staining intensity of the cytoplasm. The basal membrane is much thickened by concentrically arranged lamellæ and spindle cells; there is also an increase in the connective tissue between the tubules, with sometimes a slight round cell infiltration. The atrophy and azoospermia was complete in 24 of the 37, including 7 who died of cirrhosis; in but 4 was the change of only insignificant degree. Although similar changes of slight degree were found in non-alcoholics, the advanced changes were seen only in those who drank heavily. Even in senility the atrophy and fibrosis were less marked than in the alcoholics.

Since the reports of Simmonds and Bertholet, a few other papers have appeared corroborating their observations in all essentials, although not all investigators on alcoholism have considered the sex cells. Thus Fahr,[8] in his study of the anatomical changes in 309 alcoholics, makes no mention of the generative tract or the occurrence of changes in these tissues in the guinea pigs that he subjected to experimental alcoholism.

Weichselbaum and Kyrle[9] (1912) have furnished one of the best contributions to this subject, having carefully studied the testicles of 67 habitual alcoholics. They entirely support Simmonds' and Bertholet's conclusion that chronic

[6] Simmonds, *Berl. klin. Woch.*, 1898, xxxv, 806.

[7] Bertholet, E., *Centr. allg. Path. u. path. Anat.*, 1909, xx, 1062.

[8] Fahr, *Verhandl. deutsch. path. Ges.*, 1909, xiii, 162.

[9] Weichselbaum, A., and Kyrle, J., *Sitzungsb.-Ber. Akad. Wien.*, 1912, cxxi, 51.

alcoholism constantly causes retrogressive changes in the testicles independent of senility or chronic diseases, even to the point of complete loss of the seminal epithelium. Also they corroborate the observation that the most marked changes occur in the testicles in alcoholism with cirrhosis. They too find that tuberculosis and cancer may produce similar changes, usually of mild degree, but alcoholism of itself produces marked changes without the coexistence of any other disease. Their paper contains a detailed description of the histological changes observed.

Because of the repeated observation that alcoholics with cirrhosis show especially marked changes in the testicles, Schopper[10] investigated the effect of liver injury on the testicles. He found that animals (rabbits, dogs, and rats) from which a large portion of the liver had been removed, or in which the liver had been injured by exposure to Roentgen rays, exhibited marked changes in the testicles. These changes consisted in a cessation of spermatogenesis, transudation into the interstitial tissue, desquamation of the epithelial cells with formation of multinucleated cells, and even complete loss of the epithelium with increase in the interstitial cells.

In connection with his studies on the effect of parental alcoholism on the development of the offspring, Stockard[11] stated that guinea pigs given alcohol for as long as 19 months by inhalation, and therefore in unknown quantity, were still good breeders, and when killed showed no changes in the testicles. Ovaries of females similarly treated also showed no recognizable structural changes. On the other hand, Riddle and Basset[12] found that doves and pigeons made to inhale alcohol 1 to 2 hours daily for a few weeks produced eggs with yolks smaller than those in the eggs laid before the alcoholism. In general, however, the possible anatomical changes in the ovaries seem not to have been considered by pathologists, and the only mention we can find of this topic is contained in the review of Forel,[13] who says, "more recently Bertholet has also demonstrated atrophy of the ovaries and ova of an alcoholic woman," but no reference is given and we have been unable to locate this statement in Bertholet's writings.

The only investigation of the influence of experimental alcoholism on the testicles of animals that we can find, beyond Stockard's negative results, is described by Bouin and Garnier,[14] and unfortunately this report concerns only an isolated observation. It is stated by these authors that in the course of experiments on the effect of chronic alcoholism on the white rat, marked alterations were observed in the testicles of two animals that had been fed alcohol for 8 and 11 months respectively. No mention is made of how many animals were studied in

[10] Schopper, K. J., *Frankfürter Z. Path.*, 1911, viii, 169.

[11] Stockard, C. R., *Arch. Int. Med.*, 1912, x, 374; *Arch. Entwcklngsmechn. Organ.*, 1912–13, xxxv, 3. Stockard, C. R., and Papanicolaou, G., *Am. Naturalist*, 1916, i, 65.

[12] Riddle, O., and Basset, G. C., *Am. J. Physiol.*, 1916, xli, 425.

[13] Forel, A., *Münch. med. Woch.*, 1911, lviii, 2596.

[14] Bouin, P., and Garnier, C., *Compt. rend. Soc. biol.*, 1900, lii, 23.

ail, or whether the other animals showed any changes in the testicles. In these two animals there was marked atrophy of the seminal tubules, often with almost complete disappearance of the germinal epithelium, and in none was the epithelium normal. Between the tubules was found an accumulation of lymph, but no fibrosis or arteriosclerosis. In the lumen of the tubules few spermatozoa were found, but there was much desquamation of the epithelium, and often fusion of the cells into masses. Pyknosis and karyolysis were common, and the Sertoli cells often showed amitotic division. Before degenerating completely the germinal cells pass through various stages of altered vitality, which, the authors suggest, explains the frequency of abnormalities in the offspring of alcoholics. We cannot find that these authors have published elsewhere further details of their investigations in this field.

General Physical Changes Due to Alcohol.

In a series of rats fed alcohol for varying periods of time to determine the effect of alcohol on the psychology of their offspring, an opportunity was given to observe the effect of chronic alcoholism on these animals.[15] Altogether we examined 15 male rats thus treated, 6 male rats of the second generation derived from them, 18 male rats of the third generation, and 8 male rats of the fourth generation. Approximately the same number of females was also studied. From each animal, with a few exceptions, the following organs were studied: lung, liver, kidney, spleen, stomach, heart, and sex glands. The animals were all killed, while in apparent health, with illuminating gas. They were all of about the same age, 6 to 9 months, except in the case of the rats fed 0.5 cc. for 10 months; these rats were 13 months old. All had been fed and cared for under identical conditions except for the administration of alcohol admixed with the food in quantities of from 0.25 cc. to 2.25 cc. per day, for periods varying from 2 to 10 months. The effect of the alcohol on the rats receiving it was, except for those fed for very long periods, to lessen learning capacity in almost direct proportion to the amount of the dose and the duration of the period during which it was administered.

The effect on body weight was also marked. Animals receiving 2.25 cc. *per diem* rate either gained weight far more slowly than the normal animals, or, as in some instances, actually lost weight though in the

[15] The results of the psychological study will be published shortly in *Psychol. Monog. Suppl.*

growing period. As for the offspring, the observed physical effects were, in the case of the offspring of rats receiving 0.5 cc., a decrease in size and lessened fertility. This was accompanied by a marked decrease in learning capacity. The offspring of animals receiving 0.25 cc. showed no marked physical changes and were, as to intelligent behavior, generally on a par with, if not actually superior to, the offspring of normal animals.

The microscopic changes in all structures except the sex glands may be summarized briefly. Nearly all showed more or less pulmonary infection of the type common in rats, consisting in the less advanced stages of a chronic bronchitis with much cellular infiltration and hyperplasia of the bronchial walls. Many showed extension of the process to the adjacent alveoli with a frank mucopurulent bronchopneumonia. The degree of this change seemed to have little effect on the conditions in the other organs, and bore no relation whatever to the changes observed in the sex glands.

In the liver there were often varying degrees of hydropic vacuolization of cells, this condition occurring in about half the specimens. Focal necrosis was found in about one-fourth, severe in but a few. These changes bore no reference to the severity of the lung infection or to whether alcohol had been administered, as shown by tabulation and comparison of the results. Occasionally a slight periportal round cell infiltration was found, but this also was independent of other conditions. Even in the rats that received large quantities of alcohol for some months, the livers showed no more change than in the others. There was nothing found at all resembling cirrhosis.

The kidneys showed commonly a slight degree of vacuolization of the tubular epithelium, which was possibly slightly more general in the alcohol animals than in the others. Two animals in which the kidneys showed slight formation of granular casts had received much alcohol. That the alcohol was the cause of the renal changes is probable, especially in view of the great amount of cast formation commonly observed in the urine of patients with acute alcoholism, but this is by no means certain.

In the stomachs of a few of the rats receiving alcohol there seemed to be slightly more round cells in the mucosa and submucosa than normally, but the changes were not marked enough to be considered as

distinctly inflammatory. In two of the alcoholized rats the gastric glands showed more mucus content than was seen in any of the other animals, and hence this change may be ascribable to the alcohol. The slight effect of the alcohol on the stomach of the rats corresponds to the relatively normal condition of the gastric mucosa of human alcoholics observed by Hirsch.[16]

The heart, spleen, and adrenals usually showed no changes, and in no instance changes that occurred in the alcoholized rats and not in the others.

Pathological Changes in the Reproductive Tissues.

Only the testicles showed definite changes, but here the effects were so marked, and so nearly constant, that they stand out conspicuously as the result of the alcohol feeding. Of the fifteen male rats treated with alcohol, the testicles of not more than two or three failed to show noteworthy changes, and only one could be called normal. Several different types of changes were observed, and one that attracted attention at once was an apparent decrease in the size of the seminiferous tubules, and in several instances a distinct decrease in the size of the testicle itself. Measurements were made of the tubules in the testicles from thirty-seven rats, twenty-five tubules being measured from each testicle to secure adequate representation, and then the average diameter was calculated.[17] The results of measurements in the several groups are given in Table I.

TABLE I.

No. of animals.	Minimum diameter.	Maximum diameter.	Average diameter.
6, 2nd generation rats	16.00*	19.16	17.26
18, 3rd " "	15.08†	22.60	17.50
8, 4th " "	15.56	18.32	17.08
15, alcoholized rats	10.00	18.24	14.65

* The measurements as recorded are the number of ocular micrometer spaces, not translated into microns. Each ocular micrometer space = 0.0155 mm.

† One very abnormal testicle in this series, measuring but 12.12, is not included.

[16] Hirsch, E. F., *Arch. Int. Med.*, 1916, xvii, 354.

[17] For these measurements we are indebted to Miss C. L. Chapin.

These measurements show at once that there is a distinct decrease in the size of the seminal tubules of the rats that had received alcohol, although in a few rats this change is not present. However, as Table II shows, but two of the fifteen rats that received alcohol had as large tubules as the average seminal tubules of their descendants that had not received alcohol. It also shows that the decrease in size does not vary directly with the amount of alcohol the rat had received. The tubules of normal rats of other strains corresponded in size to the tubules of the second, third, and fourth generation rats.

TABLE II.

Rat No.	Daily dose.	Time.	Diameter of tubules.	Histological changes summarized.
	cc.	*mos*		
1	0.25	2	13.40	Many defective spermatozoa; number decreased.
2	0.25	2	15.04	Spermatozoa decreased.
3	0.25	4	13.68	Few spermatozoa; mostly defective.
4	0.5	3	17.12	Nearly normal.
5	0.5	3	16.16	A few inactive tubules; mostly normal.
6	0.5	3	18.24	" " " " " "
7	0.5	10	11.32	Marked atrophy with intertubular edema; almost no spermatozoa, and these defective.
8	0.5	10	16.76	Much intertubular edema; most of tubules nearly normal, but number of spermatozoa decreased.
9	2.0	6	14.32	Very few spermatozoa; much desquamation.
10	2.0	6	14.16	Much desquamation and degeneration of epithelium; small number of spermatozoa.
11	2.25	3	10.00	No spermatozoa; great desquamation and degeneration of epithelium.
12	2.25	3	13.56	Only one-third to one-half the tubules contain spermatozoa, and few of these are normal.
13	2.25	6	15.84	Some edema; spermatogenesis much reduced.
14	2.25	6	13.88	Much atrophy and edema; spermatogenesis about 50 per cent of normal.
15	2.25	6	16.24	About three-fourths of the tubules nearly normal.

From Table II it will be seen that the least changes were found in the three rats (Nos. 4, 5, and 6) that received 0.5 cc. of alcohol for 3 months, while much more marked changes occurred in the three animals that received but 0.25 cc. of alcohol. Also, three of the rats that received the relatively enormous dosage of 2.25 cc. per day for

6 months, which would be equivalent to a man's taking over 2 quarts of whisky daily for a large part of his life, showed less change in the testicles than some of the rats that received somewhat less alcohol.

The changes produced by alcohol seem to take place in definite order. At first the spermatocytes seem to be normal in number and appearance, but there is soon an increase in the number of spermatids in the tubules with a decrease in the number of spermatozoa (see Fig. 1, showing a normal testicle, and Figs. 2 and 3, showing the testicles of rats which received 2 cc. and 2.5 cc. respectively). At the same time, or earlier, there is observed a greater diminution in the number of complete spermatozoa with tails than in the number of sperm heads. Not all the tubules in the testicles show the same degree of change, except in the most advanced cases (Fig. 4). In the more moderately affected testicles one finds some tubules nearly or quite normal, while others show merely a few active spermatocytes with the lumen packed with spermatids without spermatozoa or even spermatozoa heads (Fig. 5). Apparently the first effect of the alcohol is to render the formation of spermatozoa incomplete, so that the heads are formed without normal tails. In accordance with this, in one of these testicles we usually find the number of spermatozoa in the tubules of the epididymis much less than normal, and less in proportion than the number of spermatozoa seen in the seminiferous tubules, suggesting that the latter are too imperfect to migrate to the epididymis. The next effect of the alcohol seems to be to prevent the transformation of the spermatids into spermatozoa, whereby the tubules become filled with accumulated spermatids, with but a few spermatozoa or none at all. These spermatids then undergo a process of degeneration, so that they lose their nuclear stain and become granular. In the most advanced stages the tubules contain but the marginal cells, with few or no spermatocytes or spermatids (Fig. 6). Occasionally in the lumen of tubules showing advanced degeneration are found large cells with many nuclei or chromatin masses.

The degree of atrophy does not exactly parallel the decrease in functional activity, but usually the diameter of the tubules corresponds somewhat with the loss of function. When the tubules are atrophied there is commonly some compensatory accumulation of

serous fluid in the interlobular spaces, an edema *ex vacuo* (Fig. 4). In no instance have inflammatory changes been seen, and no marked fibrosis, although in some instances there has been an apparent slight increase in the thickness of the basement membrane of the tubules. We have noted no definite multiplication or other changes in the interstitial cells of Leydig, although when the tubules shrink apart these cells become more evident. No arterial changes were observed.

The epididymis generally reflects, in the number of spermatozoa it contains, the degree of functional impairment of the testicle (Figs. 7 and 8). In two of the rats there was also noteworthy desquamation of the epithelial lining of the ducts. Other changes were not observed in the epididymis.

Although the ovaries of the alcoholized females, and of the offspring of alcoholized parents, were examined in considerable numbers, it is not possible to make any positive statement as to the effect of alcohol on this organ. The changes of ovulation, pregnancy, and corpus luteum formation produce such great structural alterations in the ovary that we were unable to determine satisfactorily what changes were caused by the alcohol. There were two distinctly atrophic ovaries found in females that had had alcohol, and in several alcoholic females the ovaries were poor in ova in the sections examined.

Without study of serial sections of every ovary no conclusions of value could be reached from the material, and we were unable to undertake this task.

SUMMARY.

Administration of alcohol in the food of male white rats for 2 or more months, in daily quantities of 0.25 to 2.25 cc., results almost constantly in the appearance of marked degenerative alterations in the testicles. These changes affect the steps of spermatogenesis in inverse order to their occurrence, so that for some time before sterility and complete aspermia result, the animal is producing spermatozoa with all possible degrees of abnormality and deficiency. The possible relation of this abnormal spermatogenesis to the production of defective offspring is obvious. Individual rats show marked differences in the degree of change produced by equal amounts of alcohol. The

fibrous, interstitial, and vascular elements of the testicle are not affected, except for intertubular edema compensating for tubular atrophy. These experimental observations harmonize with the necropsy findings in human alcoholics. No other tissue was found to be noticeably affected by the alcohol; especially to be remarked is the absence of cirrhosis or fatty infiltration in the liver.

EXPLANATION OF PLATES.

Plate 57.

Fig. 1. Normal testicle of a rat. × 58.

Fig. 2. Testicle of a rat which had received 2 cc. of alcohol daily for 6 months. Marked loss of germinative epithelium and absence of spermatogenesis are shown, without much decrease in size. Many tubules in this testicle also show spermatogenesis. × 58.

Plate 58.

Fig. 3. Testicle of a rat which had received 2.25 cc. of alcohol daily for 3 months. Marked atrophy of the cellular content, decrease in size of the tubules as a whole, and complete absence of spermatogenesis are shown. × 58.

See higher power of the same in Fig. 6.

Fig. 4. Testicle of a rat which had received 0.5 cc. of alcohol daily for 10 months. Marked decrease in size of the tubules with intertubular edema. × 58.

Plate 59.

Fig. 5. Tubule of a rat which had received 0.25 cc. of alcohol daily, showing absence of spermatozoa and accumulation of spermatids. This is one of the early stages of the degeneration of the testicular tubules. × 309.

Fig. 6. Higher power of Fig. 3. Later stage of degeneration. The tubules contain no spermatozoa or spermatids, and almost no spermatocytes. × 309.

Plate 60.

Fig. 7. Normal epididymis of a rat. Tubules filled with spermatozoa. × 58.

Fig. 8. Epididymis of a rat which had received 2 cc. of alcohol for 6 months, showing great atrophy and no normal spermatozoa. × 58.

FIG. 1.

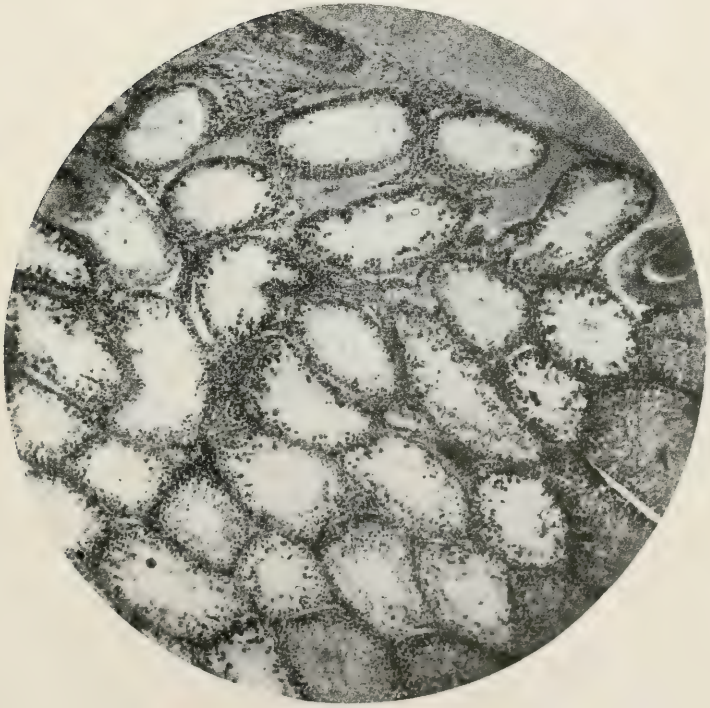

FIG. 2.

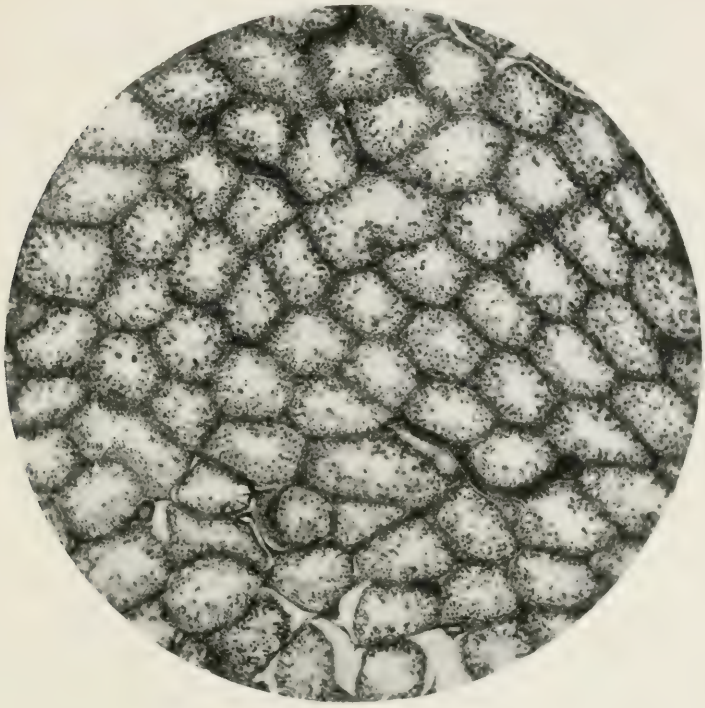

FIG. 3.

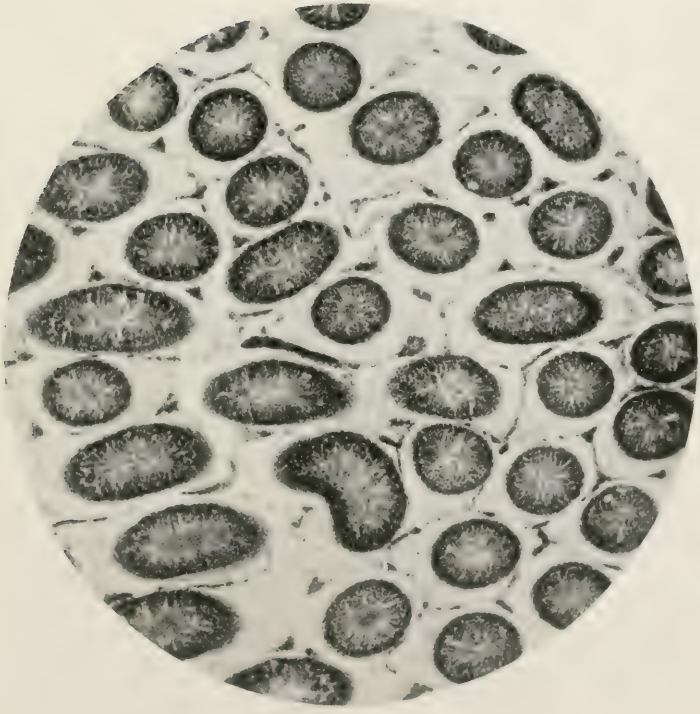

FIG. 4.

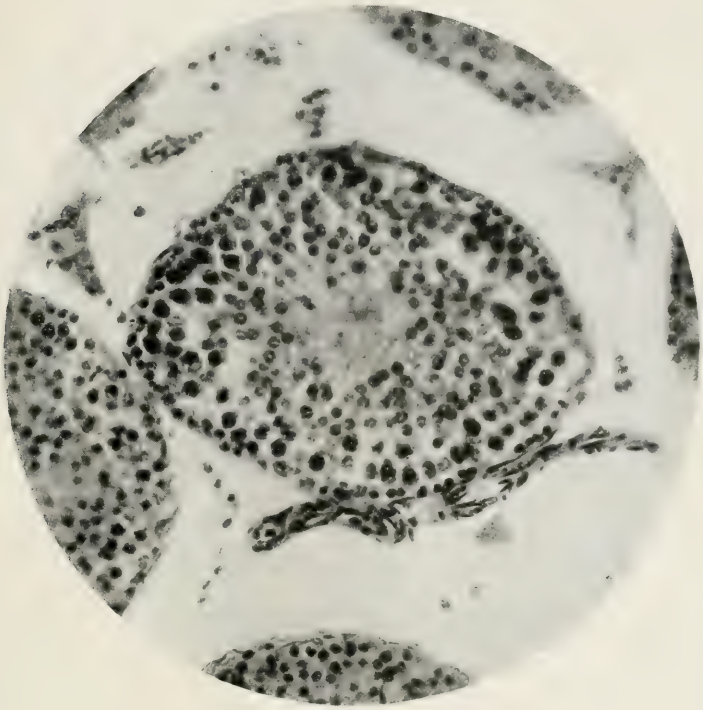

FIG. 5.

FIG. 6.

(Arlitt and Wells: Effect of alcohol on reproductive tissues.)

FIG. 7.

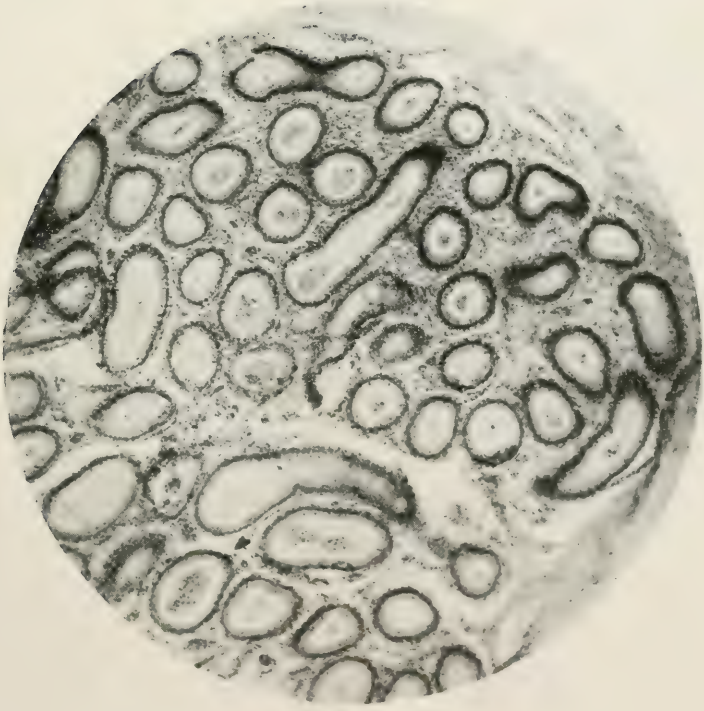

FIG. 8.

(Arlitt and Wells: Effect of alcohol on reproductive tissues.)

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THE METHOD OF TIMED INTRAVENOUS INJECTIONS.

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Plates 2 and 3.

(Received for publication, February 1, 1917.)

INTRODUCTION.

If a soluble diffusible substance, such as a sugar, an amino-acid, or a salt, is made to enter the living organism at a constant rate for a long enough time, the tendency is toward the establishment of an equilibrium which is manifested by a constancy of the rate at which the organism rids itself of the substance as such, by excretion, or chemical change, or both. The rate of entry is then equalled by the rate of utilization plus the rate of elimination.

Since, during the maintenance of a uniform entry rate, the velocity of excretion cannot vary independently without affecting the quantity of substance left over in the body for utilization and *vice versa*, the rate of utilization cannot vary independently without affecting the quantity left for excretion, and since it is known that the rates of utilization and excretion are influenced by the amount of available substance, so the occurrence either of a constant rate of elimination or a constant rate of metabolism may be used as an index of equilibrium. Now if a substance of the character mentioned is made to enter the body of a higher animal or man at a constant rate, A, until equilibrium is established, observations may then be made on the rates at which various metabolic or other physiologic processes proceed during the period of equilibrium. If then under parallel conditions the same substance is introduced at a second rate, B, bearing to A some known numerical relationship, a second equilibrium may be established with a corresponding set of observations, and so on. In this way relatively exact quantitative data may be ob-

tained of a type which should favor the interpretation of more biological processes in terms of quantitative chemistry. We have in mind especially dissociation and the laws of chemical equilibrium. In a similar way we may compare the effects produced by bringing two different substances separately into the body at the same rate, or find the rates at which two or more substances have to be given in order to produce an identical arbitrarily selected effect.

Rates and Concentration.—To establish an equilibrium during the entrance of a given substance into a given animal at a given rate naturally implies the creation of some constant concentration of the substance in the body as a whole, *provided, however, the volume of the body is kept constant*—a condition which is not fulfilled, for instance, when in spite of the establishment of an equilibrium for one substance such as glucose, there is no equilibrium with respect to another such as water. What absolute concentration is actually produced must depend on many variables, such as the character and state of the substance, the species of animal, its physiological condition, the physicochemical state of its tissues, etc. This absolute value could only be found by direct experiment for any specific case. To establish a constant concentration in the body as a whole would also imply, of course, a tendency toward the establishment of constant concentrations in each of the several tissues or body media corresponding to the relative solubilities of the substance in each, in accordance with laws which pertain to the partition of dissolved diffusoids among the phases of a heterogeneous system. The absolute values of these could also be found, if at all, only by direct observation in specific cases. *But for the purposes outlined above it is not necessary to know what concentrations are attained either in the body as a whole or in any of its parts,* although under conditions otherwise the same it might be assumed that they would vary in proportion to the rates at which the substances entered the body. In the case of simpler marine forms it is a common practice to control the rate at which an experimental substance enters the organism *by fixing the concentration of the substance in the circumambient liquid.* If the latter is changed frequently enough, or if its volume is made sufficiently great in proportion to the mass of the cells, then once the latter have ap-

proached equilibrium with the environment, molecules of the experimental substance migrate into the organism at a rate fixed by the concentration. With a gas, the concentration of which in the water is proportional to its volume percentage in the atmosphere over the water, the rate at which the substance enters the organism may be controlled by fixing its concentration in the air. And this method is available in the case of higher animals when the experimental substance is volatile like chloroform and ether, or a gas like oxygen or carbon dioxide, for the sea water then has its counterpart in the blood. The application of these principles in respiration studies by numerous workers has already led to notable achievements. *But in the case of higher animals and non-volatile substances regulation of the entry rate through the expedient merely of fixing a concentration is impractical, and recourse must be had to direct control of the entry rate into the blood, leaving the concentrations to adjust themselves.*

For well known reasons it is not possible to administer a substance by alimentary, subcutaneous, or other indirect routes and directly control the rates at which it will be taken up into the blood. On the other hand, if it is injected directly into a vein, the mechanical difficulty arises of sustaining the injection at a uniform rate for a long enough time. The problem of controlling the rate at which non-volatile substances are brought into the bodies of higher animals then resolves itself into one of mechanical apparatus.

Requirements for Apparatus.

A satisfactory apparatus should embody as many as possible of the following specifications. *In general,* it should be an instrument of precision, compact, portable, quiet in operation, for use by the bedside or in the laboratory. *The range of possible delivery rates* should be so wide as to meet the needs of experiments in laboratory animals and man. *The rate control* should be convenient and rapid, permitting of coarse and fine adjustments through a working range during the progress of a single experiment without interruption, and should allow a ready adaptation of the apparatus to the requirements of different experiments in which the ranges of rate differ widely. *An automatic rate-registering device* to indicate at every moment the actual delivery rate would be highly

desirable, and to this end the entire action of the mechanism should be positive so that the pump, when running at any particular speed shall inevitably move a corresponding volume of liquid. *The power and uniformity of action* should be such that a selected delivery rate may be sustained uniformly for 2 to 24 hours or more, with little attention in spite of variations of resistance such as may arise from changes of blood pressure or partial occlusions of tubing, cannulas, needles, or veins. *The accuracy* should be such that uncontrollable errors in the rate of delivery shall fall within the limits of error of methods available for detecting the physiological effects of such variations.

Parts coming into contact with the injection fluid should be quickly removable and replaceable without tools, for cleansing and sterilization. *The materials* should be selected with a consideration of their solubility in various injection media and their possible influence on the organism. Complex or delicate adjustments, valves, or other parts requiring undue care, or likely to become deranged or lost during ordinary use or cleaning, should be avoided.

Existing Types of Apparatus.

The various forms of apparatus described in the literature fall into three classes: (A) Devices in which pressure is imparted by gravity alone and the rate corrected by varying the size of the outlet. (B) Those in which pressure is imparted by a chamber of compressed air and the rate controlled as above. (C) Motor driven pumps.

It is unnecessary to consider in detail the disadvantageous features of apparatus of the first two types. The most notable use of a gravity device for prolonged intravenous injections was in the experiment of Henriques and Anderson,[1] who perfused a goat uninterruptedly for 20 days with a fair degree of accuracy For a meter they used an ordinary dropping device inserted high up in the tube leading from the reservoir, a system which would not permit of a wide rate variation nor a change in the character of the liquid without recalibration. The principle of gravity flow, however refined and perfected, will necessarily involve

[1] Henriques, V., and Anderson, A. C., *Z. physiol. Chem.*, 1913, lxxxviii, 357.

cumbersome apparatus and considerable inconvenience, recommending itself only in special situations. Apparatus of Type B has been described by Kretschmer,[2] Rohde,[3] and others. Bulk, complexity, inconvenience, the undesirable solution of gases under pressure in the perfusion liquid, imperfect rate control, etc., are among their drawbacks. Motor driven pumps designed especially for organ perfusion work have been described by Brodie, Mandel, Friedmann, Straub, Kingsbury, and others. The problem of organ perfusion is not, however, identical with that of timed intravenous injections into the living animal. For perfusion purposes attention has been given especially to the injection of minute volumes of liquid in a pulsating stream, and especially by Kingsbury to materials and the form of the pulse wave. For the present problem, portability, adaptability to varying laboratory and bedside conditions, the convenient separation of parts for cleansing and sterilization, and above all positive, rapid, and exact regulation of rates through a wide range (*e.g.*, 10 to 2,000 cc. hourly), are features of prime importance. In the designs of perfusion pumps emphasis has not been laid on these points. On the other hand, the pulse form is of less significance for intravenous work.

Bock[4] was probably the first to use a motor driven pump for timed intravenous injections. His apparatus consisted of two glass syringes mounted side by side to form a duplex pump. The stems of the syringe plungers were screws and each passed through the center of a gear-wheel, as a bolt is screwed through a nut. When rotated, these wheels screwed the pistons in or out of the syringe barrels according to the directions of their rotation. The gear-wheels were turned in opposite directions by a single pinion engaging between them. Power was applied to the pinion by an electrical motor acting through a system of pulleys and belts. When either of the piston rods was screwed out to the end of a stroke it made an electrical contact, reversing the direction of the motor, and by the same device, a double three-way valve lying in front of the syringe barrels was operated by electromagnets. This complex apparatus delivered 0.2 to 10 cc. per

[2] Kretschmer, W., *Arch. exp. Path. u. Pharm.*, 1907, lvii, 428.
[3] Rohde, E., *Z. biol. Tech. u. Method.*, 1913, iii, 85.
Bock, J., *Arch. exp. Path. u. Pharm.*, 1907, lvii, 177.

minute and was used in a study of renal function. The principle of reversing the motion of the pistons through the motor together with the belt transmission would necessarily involve irregularity and slippage. In 1913 Friedenthal and Willner demonstrated at the International Congress of Physiologists in Gröningen, a "*Praezisionsinjectorium*," concerning the details of which we have at present only the following program abstract: "The instrument serves the purpose of making possible the injection of specified solutions in the time desired and at the rate wished. It allows several experiments to be conducted simultaneously, or the injection of several solutions at different places into one animal. It permits further the preparation of unstable mixtures directly before injection. The instrument is also as appropriate for the most beautiful anatomic injections as it is for physiological and especially pharmacological and therapeutic injections." This is perhaps a later development of one of Friedmann's earlier perfusion pumps with multiple cylinders operated by a single machine, described in 1910.[5] an arrangement which would not be selected for the greatest exactness of rate control. In a report published in 1915[6] on the effects of prolonged and accurately timed intravenous injections of sugar are a cut and a brief description of a machine which has been used in this laboratory, consisting of a glass barrel and a metal piston driven by an electrical motor through worm and gear with a mechanically operated valve and a rheostat control. The model to be described presently represents merely a refinement of this machine. Raeder[7] has also mentioned using a motor driven Record syringe with a Bunsen valve to maintain even injections of salt solutions in a study of respiratory metabolism. The following cuts and descriptions are of an instrument built for us by William Gaertner and Company, of Chicago. Mr. Gaertner has embodied in it original solutions of some of the mechanical problems presented, such, for instance, as the link motion to meet the demand for a rapid means of adjusting the stroke of the pump, a feature which greatly increases the comfort of operation.

[5] Friedmann, E., *Biochem. Z.*, 1910, xxvii, 87.

[6] Woodyatt, R. T., Sansum, W. D., and Wilder, R. M., *J. Am.. Med. Assn.*, 1915, lxv, 2067.

[7] Raeder, J. C., *Biochem. Z.*, 1915, lxix, 257.

New Apparatus.

The apparatus as a whole comprises two elements: (*a*) the pump and (*b*) the driving machine. Fig. 1 shows the pump unit taken apart for sterilization. Fig. 2 shows the same assembled and ready for insertion *en bloc* into the machine. Fig. 3 is a view of the machine from directly above showing the pump to one side of its bed on the driving mechanism in the position it would assume during detachment or replacement.

The Pump.—The pump (Figs. 1 and 2) consists of a metal piston with its rod, a ground glass cylinder, and a valve. On the extremity of the piston rod is seen the block by which the piston rod when in use is coupled to the carrier block of the driving machine (C in Fig. 3). The ground glass cylinder consists in this case of a 2 cc. Record syringe barrel with a special tip. The valve is seen to consist of a metal block perforated with a tapering hole and a tapering German silver core to fit. The principle of the valve, which is that of a three-way glass cock, is apparent from the cuts. In the center of the smaller end of the valve core is a screw projecting in the direction of its long axis. When the core is pushed into its place in the valve block, a spring washer is slipped over this screw and a circular milled nut screwed down to hold the block and core together with the proper tension. These details are visible in Fig. 1. On the lower end of the core, as seen in Fig. 2, is the slot to receive the valve lever by which the core is rocked to and fro, and the milled set nut to clamp the lever in place. It may be noted that the pump as a whole constitutes an interchangeable part which may vary in size and design and still be used on the same driving base. Thus, if it should be desired for special purposes to use the machine for timed injections of minute volumes of liquid, it would be possible to construct a special unit for this purpose. Since the stroke of the pump can be reduced literally to zero and the caliber of the barrel made as small as desired, the lower limit of the volume of liquid which can be pumped per minute depends wholly on the effectiveness of the valve.

The Driving Mechanism.—Power is provided by an electrical motor of $\frac{1}{20}$ horse power, and as it may be desired to use a single machine in different locations where the current available differs

in type, that shown in the cut (Fig. 3) is equipped with a universal motor which operates with any 110 to 120 volt current. For the best results, as with any motor, it is essential that the commutator be kept clean, and attention has been given to the details which make for the adequate protection and accessibility of this part. On the motor shaft in front of the motor casing is a heavy and carefully balanced leaden fly-wheel F, which adds greatly to the momentum and steadiness of the machine when running; and further forward the shaft is coupled through a flexible connecting piece with a worm running in oil on ball bearings. Only the protective housing of the worm is visible in the cut at W. Engaging with the worm is a large horizontal gear, the center of which is visible at G, while the remainder is likewise encased for protection. The gear-wheel shaft bearings are provided for in a heavy pillar which forms a part of the base casting of the machine. The gear-wheel revolutions are to those of the motor and worm as 1 to 200 (subject to change). The motion of the gear-wheel is transmitted to the piston of the pump through a system of links and levers designated on the cut as 1, 2, 3, 4, and 5, from which it is seen that one revolution of the gear-wheel corresponds to one complete oscillation of the piston. These links are so arranged that the length of the piston stroke may be set (if necessary, while the machine is in motion) at zero, 35 mm., or any intermediate value. This is effected at the lever 2 with the scale markings. The fixed pivot of this lever is beneath the end nearest the syringe under the letter S and on the lever is a sleeve (also S) which can be fixed in any position by a thumb screw. The link 3 is pivoted to the sleeve S and the length of the thrust it receives depends on the distance of the sleeve from the pivotal point of lever 2. The last link of the system 5 imparts its motion to a carrier block C which slides on a guide G. On this carrier block there is a screw with a milled head which serves to couple in place the block at the end of the pump piston when the apparatus is assembled. The valve of the pump is operated by an eccentric which lies beneath the gear-wheel. The eccentric is in fact part of the gear-wheel casting. Its motion is transmitted to the eccentric arm E (which is seen in the cut pivoted to the same pillar as the lever 4) and thence forward by a link to the valve lever proper. The left hand end of this link is visible but the valve lever does not show in the photograph.

It will be seen that in this machine the oscillations of the piston are directly proportional to the revolutions of the motor and cannot be otherwise. Also the valve is turned forcibly back and forth at the proper part of the stroke as an integral part of the motion of the whole machine. If the valve stops, the machine stops unless some part is broken or disconnected. Therefore, barring the factor of leakage in the valve and a wholly avoidable leakage about the piston or connections, the amount of liquid drawn into the pump and ejected during any interval is directly proportional to the number of strokes and this to the velocity of the motor. This would make it practical to provide the machine with a speed indicator for direct readings of the delivery rate at any moment, although this desirable factor has not been embodied in the present model. While the positive nature of the valve mechanism will naturally not insure against leakage due to imperfections in the valve itself, it has an advantage over delicate (ball, cone, or flap) valves in that the latter may be prevented from seating perfectly by gumming with thick liquids such as citrated blood, or from the lodgment of small particles between the valve surfaces. Leaks may thus develop during an experiment without affecting the speed of the machine and be detected only by timing the fall of the liquid in the supply burette. With a well made valve of the rotary type forcibly turned, this source of inconvenience is virtually eliminated and the machine becomes in reality a meter as well as a pump.

Performance.—The uniformity of the delivery rate with machines of the above design will depend practically on two factors: the perfection with which they are made, and the steadiness of the power current. An evenly balanced, smooth running motor, a true fly-wheel, well cut worm and gears, exact centerings and alignments, accurately fitted valves, proper oiling, etc., are of course essential. If these are satisfactory, the delivery rate is determined chiefly by the current and very little by variations of the pressure to be overcome in the tubing cannula, needle, or blood vessels. Two machines have now been subjected to heavy service in the wards and laboratory for a period of 12 months. One of them runs with great regularity and very quietly; the other is somewhat less quiet and requires more attention in order to secure even performances. They will aspirate liquids of vary-

ing viscosities such as water, citrated blood, or glycerol of 1.26 specific gravity, from burettes, flasks, beakers, etc., placed above or below them, and develop sufficient pressure to burst an ordinary rubber delivery tube if the latter is clamped while the machine is running, and this without materially slowing the rate of the machine. The stream emerging from an open delivery tube intermits twenty to forty times per minute according to the number of strokes. When the delivery tube terminates in a needle or cannula, the elasticity of the wall tends to equalize the pressure and an undulating stream results. The stream can be made continuous and uniform by the use of a section of thin-walled tubing with a narrow outlet or other equalizing device. For intravenous work it has proved satisfactory. A single machine has been run continuously for 19 hours by Sansum in actual animal experimentation, and they might be run for days. It has not been found difficult to make the quarter hour and hour discharge fall within 1 per cent of the desired values. With a favorable current and perfectly constructed machine, no attention whatsoever may be required for hours at a time to secure a uniform rate of delivery; but if the current fluctuates, or if the machine is imperfectly made or cared for, more attention will be necessary. By the rheostat alone the rate can be varied to a nicety between twenty and forty strokes to the minute with an ample working margin above and below. When the barrel of the pump has a bore of 8 to 9 mm., or about that of an ordinary 2 cc. all glass hypodermic syringe, the delivery rate may be varied by changing both stroke and speed from 1 cc. to 80 cc. per minute (60 to 4,800 cc. per hour). If higher rates of injection or aspiration were called for, which seems improbable, a larger barrel could be used, while the use of a barrel corresponding in size to a 0.5 cc. syringe permits of injection at rates as low as 0.2 cc. per minute (12 cc. hourly). The machine could be adapted readily for injections of smaller volumes per minute, but this has not been done with the present instruments. By the use of two (or more) machines with the discharging needle of one (or more) thrust into the delivery tube of another, or by the use of two or more machines injecting simultaneously into different sites in the same animal, a great variety of experimental combinations is made possible. We have, for example, injected a standard glucose solution at a

uniform rate with one machine, while with a second discharging into the delivery tube of the first, pure water, salt solutions, etc., have been superadded at specified rates. The power of aspirating fluids as well as injecting them, and to aspirate if desired at known rates, holds several possibilities.

There has been practically no inconvenience arising from occlusions of the needles, cannulas, and veins, except when the solution itself has been injurious to the vessel lining, even when injections have been made for long periods into small veins, such as the ear veins of a rabbit.

The present apparatus is doubtless subject to variation and further improvement. For special purposes, and where portability is not a factor, other mechanical solutions of the problem might be preferred, but the model described combines several qualifications of an ideal instrument and it has proved a valuable addition to the equipment of the ward and laboratory.[8]

EXPLANATION OF PLATES.

PLATE 2.

FIG. 1. The pump unit taken apart, as for sterilization.

FIG. 2. The parts shown in Fig. 1 assembled and ready for insertion into the driving machine.

PLATE 3.

FIG. 3. The machine from directly above, showing the pump to one side of its bed on the driving mechanism in the position it would assume during detachment or replacement.

F. Fly-Wheel.
W. Worm housing.
G. Exposed center of gear-wheel.
1, 2, 3, 4, 5. Links and levers which transmit power from gear-wheel to pump.
S. Movable sleeve on lever 2 for regulation of the length of the stroke.
C. Carrier block with post for fixation of the outer end of the pump piston.
G^1. Guide on which the carrier block slides.
E. Eccentric arm for valve operation.
P. Insertion point for electrical plug.

[8] Previous references to this pump have been made by Woodyatt, Harvey Lecture, 1916, and Wilder, R. M., and Sansum, W. D., *Arch. Int. Med.*, 1917, xix, 311. Later reports deal further with application of these principles and apparatus.

FIG. 1.

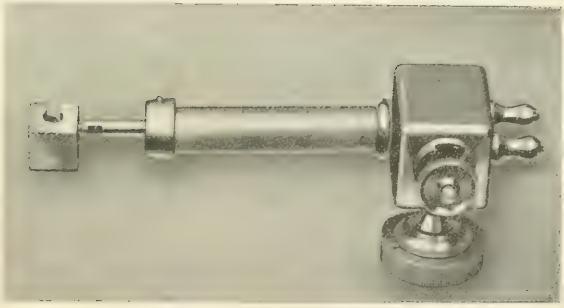

FIG. 2.

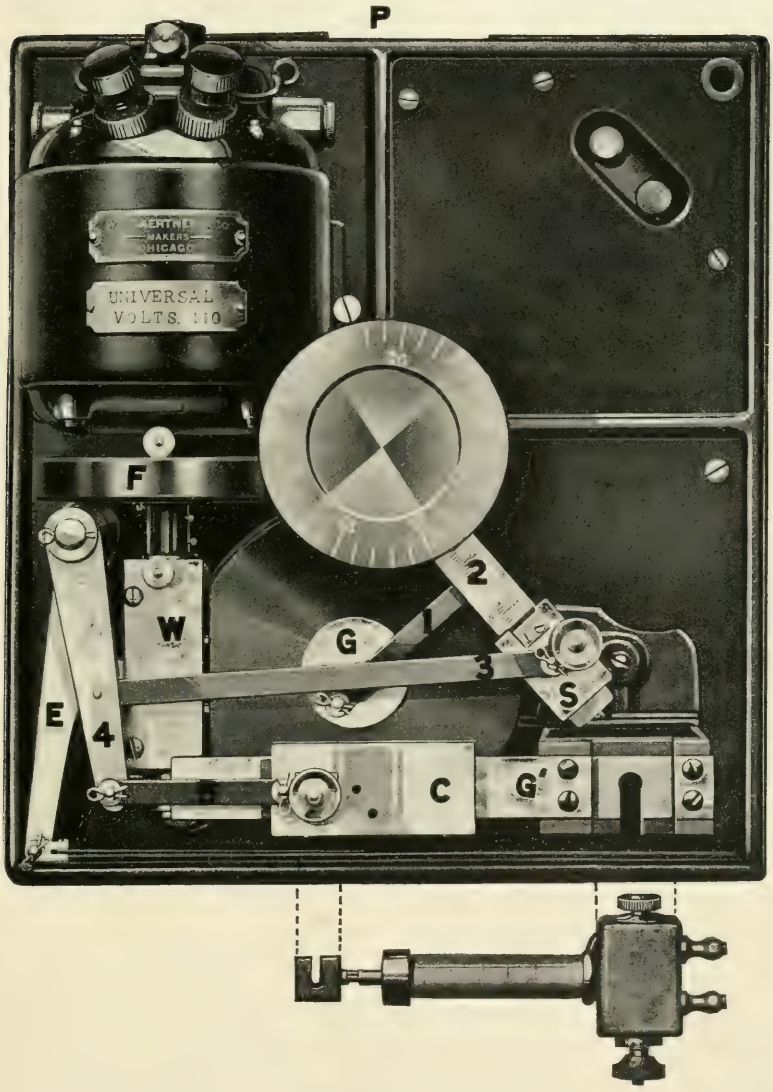

FIG. 3.

(Woodyatt: Method of Injection.)

d-GLUCOSE TOLERANCE IN HEALTH AND DISEASE*

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In previous communications[1] from this laboratory descriptions have been given of apparatus devised for the purpose of making continuous intravenous injections at accurately controlled rates. The present paper deals with the application of this apparatus and the principles of timed intravenous injection to a clinical study of glucose tolerance in normal human individuals and in a series of pathologic conditions. In order that the work may appear in its proper relationship to that of previous writers the more important contributions to the subject may be reviewed.

It has long been known that sugars administered by mouth in sufficient amounts cause melituria, but an attempt to learn the size of the dose which would accomplish this was first made by Worm Müller[2] in 1884. Two healthy men on a diet free from carbohydrate received weighed quantities of glucose, lactose and saccharose and it was found that 50 gm. of glucose, 50 gm. of saccharose or 100 gm. of lactose produced just a trace of reducing substance in the urine, while larger doses caused severe melituria.

Hofmeister[3] in 1889 conducted a more elaborate investigation with dogs. The sugar was fed by mouth and it was found that the maximum dose that could be given and just fail to cause sugar to appear in the urine was very constant for the individual dog, although widely different for different dogs. This dose he called the "assimilation limit," a term that has since been widely employed. Hofmeister is also the author of the term "tolerance," as applied to sugars, which he used in the following connection:[4]

> The diabetic of the milder type on a strictly regulated meat diet has a certain tolerance for sugars and starches, inasmuch as he only passes a saccharine urine when such substances are administered in excess of a definite limit.

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1. Woodyatt, Sansum and Wilder: Jour. Am. Med. Assn., 1915, **65,** 2067.
2. Müller, Worm: Arch. f. d. ges. Physiol., 1884, **34,** 576.
3. Hofmeister: Arch. f. exper. Path. u. Pharmakol., 1889, **25,** 240.
4. Der Diabetiker, leichterer Form zeigt, bei streng gehandhabter Fleischkost, eine gewisse Toleranz für Zucker und für Amylacien, insofern er nur bei Zufuhr derselben über eine gewisse Grenze hinaus Zuckerhaltigen Harn liefert.

It does not appear that he drew a distinction between tolerance and assimilation limits, and since this time assimilation limits, as measured by Hofmeister's method, have been interpreted as tolerance limits.

In 1895 Linossier and Roque[5] confirmed the observations of Worm Muller and Hofmeister, but proposed, as a truer measure of the assimilability (Nutzwert) of a sugar, the coefficient obtained by dividing the quantity of sugar excreted by the quantity given. The constants as found by these authors were variable, even for the same sugar; but in 1898 Gilbert and Carnot[6] obtained a fairly constant ratio of excretion to administration by giving the sugar intravenously. Inconstant proportions occurred when the injected amounts were small, but with doses between 2.5 and 10 gm. per kilogram of body weight, the ratio of grams excreted and grams injected was always about 40 to 100.

The intravenous injection of sugar is an old procedure and it has frequently been observed when different sugars were given in this way that some were excreted more freely than others. On the basis of such observations conclusions have been drawn regarding the relative utilizabilities of the various sugars. But whether these conclusions are justified on the basis of the experiments made is open to question because of the excessive variations that occur in the results of different writers and even in those of the same observer. Pavy,[7] for example, after injecting the same sugar (glucose) in doses of 0.25 gm. per kilogram of body weight in a series of rabbits, recovered in the urine of one as little as 3.1 per cent. and from another as much as 39.6 per cent. More consistent results with intravenous injections were obtained by McGuigan and Mathews,[8] who gave dilute, 0.5 per cent., sugar solution in such a manner that 5 c.c. entered every five minutes. In some cases these injections were continued for as long as seventy-seven minutes and conclusions concerning the relative ease of oxidation of the sugars were drawn from their time of appearance in the urine. Previous to this Doyon and Dufourt[9] had discovered that the *rate* of intravenous administration influenced the amount of sugar excreted and it would appear that the variation in the results obtained by the earlier writers was due to their failure to appreciate the importance of controlling the rate of injection. Doyon and Dufourt used dogs and gave to each 2 gm. of glucose per kilogram of body weight, "en un temps variable mais avec une vitesse toujours uniforme." They do not explain how they maintained such constant injection rates and simply report the

5. Linossier and Roque: Arch. de méd. expér. et d'anat. path., 1895, **7,** 228.
6. Gilbert and Carnot: Compt. rend. Soc. de biol., 1898, **50,** 330.
7. Pavy: Jour. Physiol., 1899, **24,** 479.
8. McGuigan and Mathews: Am. Jour. Physiol., 1907, **19,** 175.
9. Doyon and Dufourt: Jour. de physiol. expér., 1901, **3,** 703.

result of one typical experiment which disclosed that 98.64 per cent. of the sugar was retained if the duration of the injection was made eighty minutes, while only 85.62 per cent. remained in the body when the same quantity was injected in fifteen minutes.

In 1905 Blumenthal[10] pointed out that the utilization of sugar by the tissues is expressible only in terms that apply to velocities, for example, as unit weight of sugar, per unit weight of tissue, per unit weight of time. The rate at which sugar is brought to the tissues must therefore be controlled if it is desired to measure its rate of utilization. This necessitates precise knowledge of the rate at which it enters the blood; but when a sugar is given by any method entailing absorption as a preliminary to its entrance into the blood, the latter rate is subject to uncontrollable variations. The most nearly exact method of determining sugar tolerance must therefore consist in direct intravenous injection. These principles he applied in a series of experiments and found (1) that glucose could be rapidly injected into rabbits in doses of about 0.85 gm. per kilogram of body weight without causing melituria, but if this dose was only slightly exceeded or if within a short time after the first dose a second injection was made, intense melituria would result; (2) that a dose of sugar could be selected which could be repeatedly injected at fifteen minute intervals for indefinite periods and always just fail to appear in the urine. From these observations Blumenthal concluded that the tissues are able to remove sugar from the blood at a rapid rate up to a certain limit, that is, until they have become "saturated," but no inference concerning utilization can be drawn from the size of the dose necessary to cause saturation or the rate at which this dose is absorbed. Such a dose represents simply a saturation limit (*Sättigungsgrenze*). On the other hand, that dose whose repeated injection at fifteen minute intervals just fails to produce melituria gives a measure of utilization, a utilization limit (*Ausnutzungsgrenze*). For glucose in rabbits this was found to be between 0.15 and 0.325 gm. per kilogram of body weight per fifteen minutes, that is, from 0.6 to 1.2 gm. per kilogram per hour.

In 1906 Comessatti[11] used the Blumenthal method and demonstrated that sugar assimilation was increased by exercise. During rest, doses of 0.2 gm. per kilogram per fifteen minutes led to melituria in two rabbits, while after exercise in a treadmill doses of 0.25 gm. given in the same manner caused no glycosuria. In 1909 Loeb and Staddler,[12] with the same method, obtained figures in resting rabbits which were lower than those reported by Blumenthal (0.09 and 0.16

10. Blumenthal: Beitr. z. chem. Phys. u. Path., 1905, **6,** 329.
11. Comessatti: Beitr. z. chem. Physiol. u. Path., 1906, **9,** 67.
12. Loeb and Staddler: Arch. f. exper. Path. u. Pharmakol., 1914, **77,** 326.

gm. per kilogram of body weight per fifteen minutes, that is 0.36 and 0.64 gm. per kilogram per hour.)

There can be no doubt of the correctness of the principle stated by Blumenthal or of the insuperable difficulties which lie in the way of accurate measurement of the power of the tissues to utilize sugar when methods are used in which it is impossible to control the rate of entry of sugar into the blood. If the sugar is given by mouth, as in the Hofmeister procedure, the rate of absorption will depend on variable factors entirely beyond control, and when the subcutaneous or intraperitoneal routes are chosen the same difficulty is encountered. A tolerance limit obtained by such methods is at best only the resultant of absorption and utilization, and yet since the appearance of Blumenthal's article writers other than those quoted above have failed to appreciate the soundness of his views and continue in their adherence to absorption methods.

In clinical studies it is a common practice to give by mouth 100 gm. of d-glucose dissolved in 200 c.c. of water or lemonade and to speak of a low tolerance if this amount of sugar leads to melituria. If no melituria results when 150 or 200 gm. are fed, tolerance is considered high, but the test is by no means reliable, as many normal persons can tolerate any quantity of glucose which they can be made to take and retain, a fact which has been confirmed strikingly by the experiments of Taylor and Hutton.[13] Nevertheless, such tests have been applied in a wide variety of clinical conditions, especially in diseases of the "glands of internal secretion," and have formed the basis of extensive theory. Other methods for measuring tolerance have received little attention from clinicians. Subcutaneous injections of sugar have been made (Voit,[14] Achard[15] and others), but the pain and danger of infections which they involve have limited their general use. Intravenous sugar injections, while employed for therapeutic purposes (Kausch[16] and others) have not been used in clinical tolerance studies and the attention of clinicians seems not to have been directed to the contribution of Blumenthal.

But while Blumenthal's statement of the principles governing accurate measurement of sugar tolerance is sound and demands recognition, his technical application of these principles is capable of improvement. This is particularly true if the method is to be applied in clinical investigation. Blumenthal's method involves repeated injections at fifteen minute intervals, and the technical difficulties of performing these on time, and with assurance that no leakage occurs, are

13. Taylor and Hutton: Jour. Biol. Chem., 1916, **25,** 173.
14. Voit, F.: Deutsch. Arch. f. klin. Med., 1897, **58,** 523.
15. Achard, cited by Lepine: Le diabète sucré, Paris, 1909, p. 234.
16. Kausch: Deutsch. med. Wchnschr., 1911, **37,** 8.

very material, particularly in patients with small superficial veins. The Blumenthal procedure is furthermore insufficient to accomplish that which Blumenthal's principles demand, namely, a perfect control of the rate of administration. His injections, repeated every fifteen minutes, are not continued at a uniform rate over the fifteen minutes, but are given rapidly at the beginning of each period. Under these conditions it is clear that in the first part of each fifteen minute period, that is, during the time actually occupied by the injection, the concentration of sugar in the blood must rise as a wave and in the interval between the injections the concentration must fall. If now the injection is made more rapidly in one case than another, the crest of the wave produced will be higher, and such a wave may overflow the kidney threshold and result in melituria, even though the tolerance for the fifteen minute period is by no means exceeded. Under these conditions it is incorrect to assume that a melituria indicates that the rate of utilization for the period has been overstepped. That this objection to Blumenthal's procedure is sound is shown by figures for the tolerance of rabbits published by Blumenthal,[10] by Comessatti[11] and by Loeb and Staddler,[12] all of whom used the same method. One rabbit of Blumenthal's showed a tolerance of 0.325 gm. per kilogram of body weight per fifteen minute period, while one of Loeb and Staddler's was as low as 0.09 gm. per kilogram per fifteen minutes; and Blumenthal's own results ranged, as stated above, from 0.15 to 0.325 gm. per kilogram per fifteen minutes, a variation of over 100 per cent. It is probable that the higher figures obtained by these authors were more nearly correct than the lower ones and that the latter occurred when the injections were made too rapidly.

In order to avoid periodic waves of sugar concentration and to give no opportunity for melituria to occur until the rate of administration at all times surpasses that of utilization, it is clearly advisable to discard intermittent or discontinuous injections in favor of continuous administration at constant rates. The latter procedure was adopted in the present study, and for the purpose of giving continuous injections use was made of the apparatus referred to above. This apparatus consists of a small pump which is driven by an electric motor. The stroke of the pump may be made very short and the number of strokes per minute raised to forty or sixty, so that the stream pumped is practically continuous. Such pulsations as occur are furthermore almost obliterated by the elasticity of the rubber tubing that connects the pump with the needle in the patient's vein. The rates of injection are easily controlled and accurately regulated by means of a rheostat.

By the use of this instrument it is a simple matter to find that rate of injection which just equals the rate of utilization or the tolerance limit.

The limits for d-glucose of different dogs and rabbits tested in this way were nearly constant and approximated Blumenthal's higher figures, as might be anticipated. Reports of these animal experiments will appear in other communications, as the present paper is limited to the results obtained in human subjects.

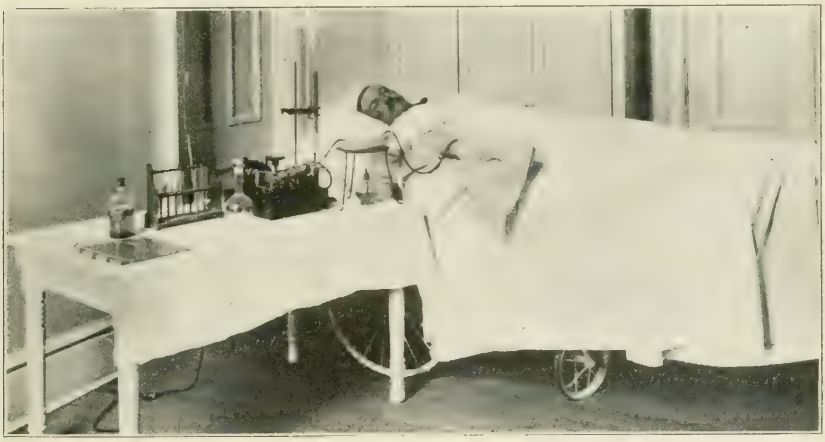
PROCEDURE IN TESTING THE d-GLUCOSE TOLERANCE OF MAN

It is to be anticipated that the intravenous tolerance limits for glucose will vary with a number of physiologic factors capable of influencing the basal metabolism. If the tolerance is expressed as grams of glucose per unit of body weight per unit of time, then sex, age, the proportion of the different types of tissue entering the body structure, the relative proportion of fat, glycogen and protein in the respective tissues, the ratio of surface to mass, the state of the activity of the muscles, etc., all require due consideration. In the present study it was found that in health both men and women showed the same tolerance. The influence of age was minimized by confining the work to adults between the ages of 20 and 45. (One child of 14 is reported.) The subjects chosen were with one exception in an average state of nutrition. All had been on a general mixed diet for several days before examination and none had partaken excessively of carbohydrates. On the day of the test no food was given by mouth and the sugar injection was commenced in the afternoon between 2 and 4 p. m. Uniform conditions of rest were secured by keeping the subjects in bed on the day of the test, and finally, in order to minimize possible delays in the excretion of glucose, once the tolerance limit had been exceeded, as well as to study the effects of different rates of glucose injection on the diuresis, water was given by mouth in amounts equivalent to the amount of fluid necessary to administer during the injection, in such a way that the subject would receive during each hour of the foreperiod an amount of water equivalent to the quantity which he was to receive by vein in each hour of the test.

Material.—The d-glucose employed in earlier experiments was a preparation of Merck labeled "purissimum." Later a high grade granular commercial dextrose (Argo corn sugar) was purified in the laboratory by decoloration with animal charcoal and repeated recrystallization from alcohol. The final product was dissolved in freshly distilled water (prepared with such precautions as are employed for salvarsan administration) to make from 16 to 20 per cent. solutions. (The molecular weight of glucose being 180, the gram molecular solution is 18 per cent.) Such a solution is sufficiently hypertonic to avoid hemolysis and has been found for other reasons to be the best standard solution to employ for the present purposes. In earlier experiments

solutions of greater strength were employed, with a view to keeping the volume injected small, but the opportunity for slight variations in the injection rate was thereby correspondingly increased and on this account the 18 per cent. solutions were adopted. The solutions showed only pale straw color when observed in flasks of two liter volume, smaller volumes appearing colorless. The solutions were divided in one and two liter flasks which were sterilized in the autoclave. The titer of each was determined by a polariscope directly before injection.

In the entire course of this work, in which the solutions were injected at rates slightly above and below the tolerance limit, no chills, shock or other general reactions were encountered. In two cases thrombosis of the vein used for the injection resulted. In one of these



Illustrating apparatus and method of making the glucose injection.

the thrombus was limited to an inch of the vein, in the other it extended to the axilla, but in neither did it result in a bad effect. It is probable that the thrombus formation in the most extensive of these cases was favored by trauma, as the subject of the test refereed a football game on the day after his injection.

Technic of Injection.—As previously stated, use is made of the injecting machine developed in this laboratory and described elsewhere. A buret to serve as a reservoir, together with the necessary rubber tubing, are sterilized by autoclave. The parts of the pump which come into contact with the fluid are removed and boiled. The apparatus is then assembled in the manner illustrated, the glucose solution is poured into the buret and all air expelled from the tube and pump.

The initial rate for injection is purposely chosen below what is thought to be the velocity of assimilation; for example, if the subject is apparently normal, it is assumed that a rate of 0.7 gm. per kilogram of body weight per hour will be tolerated, and this rate is given. If the patient weighs 75 kg. and the glucose solution, as determined by polariscope, is 20 per cent., this would mean an injection of $0.7 \times 75 \times 5$, or 262.5 c.c. of a 20 per cent. solution per hour, or 4.37 c.c. per minute. The machine is set to deliver the solution at this rate, the needle is introduced into a suitable vein and the injection begins.

This initial injection rate must be maintained long enough to permit a thorough saturation of the tissues before the urine is collected and tested for sugar. This requires from twenty to thirty minutes, as has been found by experiment. If the injection rate only slightly exceeds the rate of utilization, glycosuria will occur within this time; if it does not, no glycosuria will occur even after several hours of continuous injection. This latter statement is based on experiments with dogs. For this reason the first collection of urine is made about thirty minutes after the beginning of the experiment. One cubic centimeter of urine is added to 5 c.c. of heated Haines' solution, the mixture boiled for ten seconds and cooled and the absence of reduction is taken to indicate that the tolerance of the patient lies above the rate of injection. The injection is then accelerated and continued for thirty minutes at this higher rate, for example, at 0.8 gm. per kilogram per hour in the case under consideration. The urine is then tested again and by gradually stepping up the rate of injection, 0.1 gm. per kilogram per hour at a time, that rate can be found which first causes glycosuria and the rate just preceding this is accepted as the tolerance limit.

RESULTS

d-Glucose Tolerance in Health.—Four young adults, two men and two women, were chosen for the tests of normal glucose tolerance. All of them had been working in the medical college or the hospital and were known to be in good health. On the day preceding the test they were put to bed and subjected to the experimental conditions outlined above. The results in all these cases were the same. Glucose injected at the rate of 0.8 gm. per kilogram of body weight per hour caused no glycosuria, but in each case sugar appeared in the urine when the injection rate was 0.9 gm. per kilogram per hour. From this it would appear that the tolerance limit for d-glucose of normal men and women is practically constant and that it equals a rate of approximately 0.85 gm. per kilogram of body weight per hour of time. For a 75 kg. man such an injection would represent the administration of 63.75 gm. per hour.

Tolerance in Diseases of the Pancreas.—Three patients, one with organic disease of the pancreas and the other two with suspected mild diabetes mellitus, were tested for their glucose tolerance. In both a low limit was demonstrable. The first suffered from a chronic interstitial pancreatitis, a diagnosis which was confirmed by subsequent operation. His tolerance limit lay below 0.7 gm. per kilogram of body weight per hour. The second patient had recently shown a trace of sugar in the urine at a life insurance examination, but had been sugar free since then. His tolerance lay below 0.5 gm. per kilogram per hour. The third was similar to the second, with a tolerance of 0.4 gm. per kilogram per hour; but since the state of his nutrition and other factors besides the pancreatic disease were not strictly comparable with the controls, some reserve is expressed as to the significance of this result.

Tolerance in Diseases of the Thyroid.—Five patients with exophthalmic goiter, two men and three women, have been tested for their glucose assimilation and in all the tolerance limits were reduced. Two of these patients had severe general symptoms and these cases showed glycosuria when glucose was administered at the rate of 0.5 gm. per kilogram per hour. Two milder cases gave a limit exceeded by the administration of 0.6 gm. per kilogram per hour, and the fifth, a still milder case, had a tolerance between 0.6 and 0.7 gm. per kilogram per hour. It would appear, therefore, that a depressed glucose tolerance is a fairly constant accompaniment of exophthalmic goiter. Such results are in harmony with the observation of alimentary glycosuria in exophthalmic goiter made by Kraus and Ludwig,[17] Chvostek,[18] Falta[19] and many others and with the glycosurias which may be produced in animals and normal persons by feeding thyroid tablets (Ewald,[20] Dale,[21] Denning,[22] von Noorden[23] and others). On the other hand, a typical case of myxedema was found to have a normal tolerance, by the intravenous method, a result which is not in agreement with the observations of those who use the alimentary tests and find high assimilation limits in this disease, suggesting that a delay in the rate of absorption is accountable for their findings.

Tolerance in Disease of the Hypophysis.—Two cases of acromegaly have been studied, one case of suspected gigantism and two with dys-

17. Kraus and Ludwig: Wien. klin. Wchnschr., 1891, **4,** 898.
18. Chvostek: Wien. klin. Wchnschr., 1892, **5,** 17.
19. Falta: Die Erkrankungen der Blutdrüsen, Berlin, 1913, p. 65.
20. Ewald: Die Erkrankungen der Schilddrüsen, Myxödem und Kretinismus, Ed. 2, Leipzig, 1909.
21. Dale: Brit. Jour. Dermat., 1894, **6,** 177.
22. Denning: München. med. Wchnschr., 1895, **42,** 389.
23. Von Noorden: Ueber den Einfluss der Schilddrüsenbehandlung auf den Kohlehydratstoffwechsel, Berl. klin. Wchnschr., 1897, **34,** 518.

pituitarism, showing the Frohlich syndrome. The glucose tolerance of all these cases was found to lie close to the normal limits. Three showed a bare trace of sugar in the urine after the injection of 0.8 gm. per kilogram per hour and all gave a definite glycosuria after receiving 0.9 gm. per kilogram per hour. These results are in disagreement with those obtained when the oral administration is used (Cushing,[24] Bondi,[25] Falta[19]) and suggest, as in the case of myxedema, that the increased tolerance of such patients for sugar given by mouth is due rather to a retarded absorption from the bowel than to any anomaly of the intermediate metabolism, as certain writers have assumed.

Tolerance in Cirrhosis of the Liver.—A normal d-glucose tolerance limit was found in a case of alcoholic cirrhosis.

SUMMARY

Adopting the premise previously suggested by Doyon and Dufourt, and notably Blumenthal, that glucose tolerance must be dealt with as a velocity, that is, in terms of the number of grams of glucose which may be brought into the tissue per unit of body weight per unit of time without causing an abnormal overflow of glucose into the urine, we injected from 16 to 20 per cent. solutions of pure d-glucose intravenously at different uniform rates by means of the motor driven quantitative injection pump, which has been described in other reports from this laboratory. Observations have been made on rabbits, dogs, and normal resting men and women of average size, weight and nutrition. These experiments indicate that glycosuria appears when the rate of injection lies above 0.8 gm. and below 0.9 gm. per kilogram of body weight per hour.

It is realized that variations from these limits may be ascribed, *a priori*, to any one of several factors, namely, (1) variation in the ability of the colloids of the body at large, including those of the kidney, to hold glucose in a state of adsorption or its equivalent (variations in the threshold for glucose); (2) the variation in the rate at which glucose may be entering the cells from endogenous sources, notably glycogen and protein, but especially the former, since endogenous supplies of glucose to the cells must be regarded as superadding themselves to the glucose supplied by injection; (3) to variation in the rate at which glucose can be utilized within the cell, the utilization in this instance being taken to imply the sum of those processes by which glucose may undergo a chemical change into some substance other than glucose.

The purpose of the present report is to record objectively experi-

24. Cushing: The Pituitary Body and Its Disorders, Philadelphia, 1911.
25. Bondi: Arch. f. exper. Path. u. Pharmakol., 1910, **63**, 347.

mental findings without entering into detailed considerations of the physiologic factors which have been concerned.

Observations have been made of the intravenous glucose tolerance limit in the following clinical conditions: chronic interstitial pancreatitis, one case; very mild diabetes, two cases; thyroid disease, six cases, five representing different grades of symptoms of hyperthyroidism and one a typical instance of myxedema; hypophysis disease, five cases giving clinical pictures which would be designated as such; gigantism (?), one case; Frölich syndrome, two cases (one with diabetes insipidus and one without); acromegaly two cases; and cirrhosis of the liver, one case.

Many questions which arise as to the variations of the intravenous glucose tolerance as the result of variations in the state of nutrition, the amount of stored glycogen, the character of the previous diet, the water and salt balance of the body, etc., will be further discussed in other reports from this laboratory.

CONCLUSIONS

The following conclusions would appear to be justified:

1. Normal resting adult men and women of average size, weight and nutrition begin to excrete abnormal quantities of glucose in the urine when the injection rate into the vein is above 0.8 gm. and below 0.9 gm. per kilogram of body weight per hour.

2. Cases of nondiabetic pancreas disease may evince a lowered tolerance for glucose when measured by this method even when administration of glucose by the alimentary route gives no definite evidence of such diminution. The same is true of very mild cases of diabetes mellitus. In these cases it may be fancied that the lowered tolerance arises from a diminished utilization.

3. Cases with increased thyroid function show a uniformly diminished intravenous glucose tolerance. In such cases it is suspected that this effect may be ascribed to an increased glycogenolysis, which is tantamount to an endogenous glucose supply to the cells superimposed on the intravenous injection, or, what amounts virtually to the same thing, a decreased ability on the part of the body to build up glycogen, since increased glycogenolysis implies the disbalance between the synthesis and catalysis of glycogen in favor of the latter. On the other hand, a case with decreased thyroid function (myxedema) showed no increased glucose tolerance, but appeared to have a normal tolerance, excreting sugar promptly when this was injected at a rate faster than 0.9 gm. per kilogram of body weight per hour.

4. In none of the cases of hypophysis disease has there been any increase of the glucose tolerance above the normal limits, but on the

contrary, a tendency toward decrease, which would seem to imply that increases of glucose tolerance in such cases, following alimentary administration of glucose, are due to delays in absorption rate rather than to changes in the intermediate metabolism.

5. The d-glucose tolerance of an advanced case of alcoholic cirrhosis of the liver was found to be normal.

6. The determination of glucose tolerance by the method described is a practical clinical procedure, more accurate than those methods which depend on absorption from a local site as a preliminary to the entrance of glucose into the blood and giving more constant results than have been obtained by any other technic involving intravenous injections.

PROTOCOLS AND CASE HISTORIES

The protocols of these experiments, the case records and a composite chart of the results (Fig. 2) are appended.

CASE 1.—B. T., a healthy woman, aged 25, weight 42.7 kg., rested in bed on the day of the test.

TABLE 1.—GLUCOSE SOLUTION, 18 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.8	3.16	30	0		
0.9	3.56	30	++	0.4	0.96
Subsequent loss					0.75+
Total glycosuria					1.71+

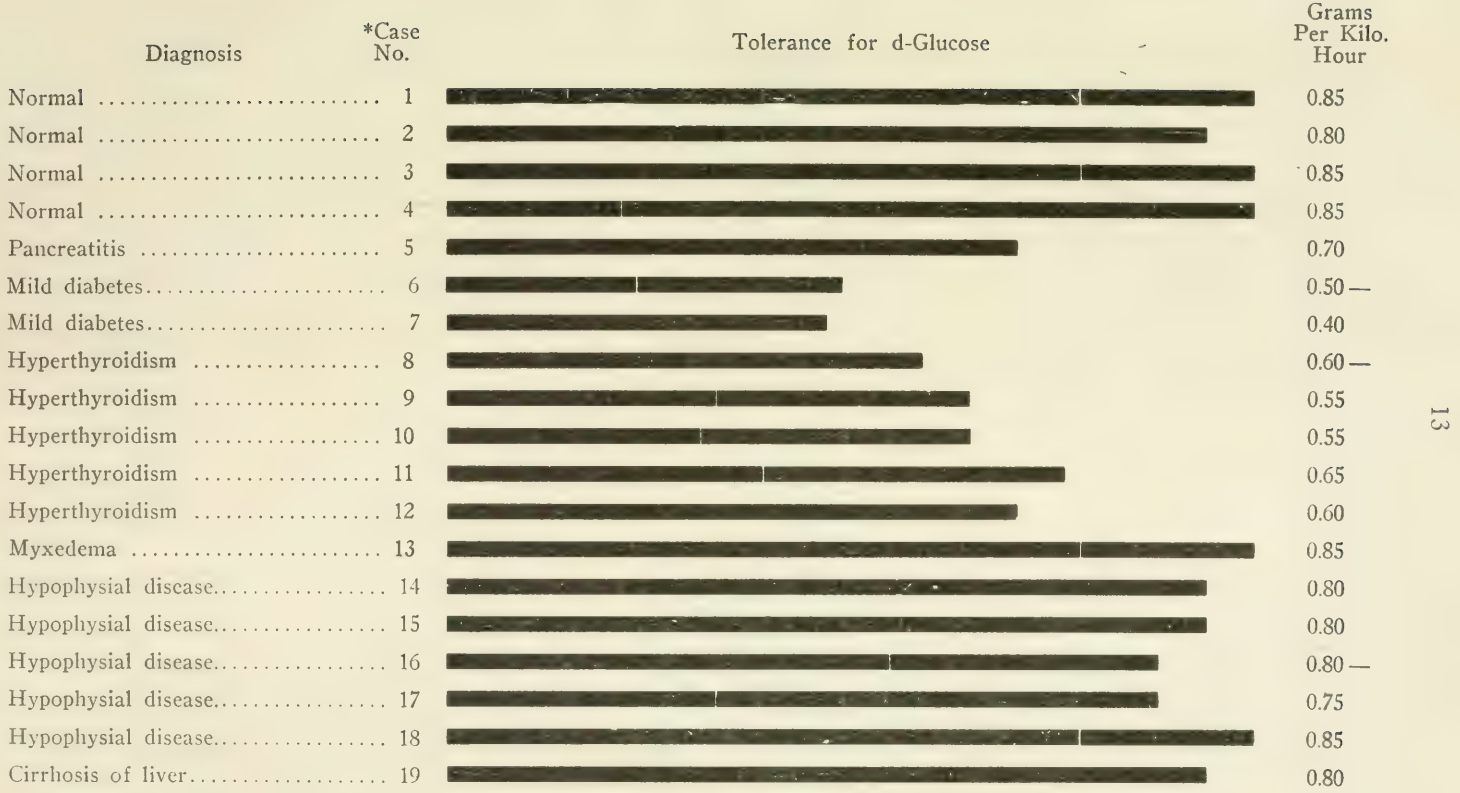
Tolerance lies between 0.8 and 0.9 gm. per kilogram per hour.

CASE 2.—P. G., a healthy woman, aged 21, weight 60 kg., rested in bed on the day of the test.

TABLE 2.—GLUCOSE SOLUTION, 18 PER CENT. BY POLARISCOPE

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.8	4.45	38	Trace*	0.16	0.77
0.9	5	20	+	0.33	1.45
Subsequent loss					?
Total glycosuria					2.22+

* During the last three minutes of injection at the 0.8 gm. rate the pump accidentally accelerated. This accounts for the trace of sugar found in the urine. The tolerance, therefore, probably lies above 0.8 but below 0.9 gm. per kilogram per hour.



*Numbers refer to case histories.

Case 3.—W. S., a healthy man, aged 35, weight 73.2 kg., rested on the day of the test.

TABLE 3.—Glucose Solution, 40.91 Per Cent.

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.85	2.38	32	Trace	0.23	0.15
0.9	2.68	30	+	0.86	1.12
				Subsequent loss	0.76
				Total glycosuria	2.03

Tolerance lies very close to 0.85 gm. per kilogram per hour.

Case 4.—J. V., a healthy man, aged 25, weight 75.45 kg., rested in bed on the day of the test.

TABLE 4.—Glucose Solution, 19.75 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.8	5.1	30	0		
0.9	5.73	30	+	0.64	1.66
				Subsequent loss	1.02
				Total glycosuria	2.68

Tolerance lies between 0.8 and 0.9 gm. per kilogram of body weight per hour.

Case 5.—Diagnosis, chronic pancreatitis. J. W., aged 45, was admitted to the service of Dr. J. B. Herrick at the Presbyterian Hospital on Nov. 6, 1915, with complaints of jaundice and intense itching. He gave a history of syphilis in 1888, for which he received treatment, herpes zoster in 1911, and in 1912 jaundice without pain, fever or chills, which lasted two weeks. In August, 1915, he again became jaundiced, this time with cold sweats, a chill and fever. There was no pain and the fever disappeared, but the jaundice continued. The patient lost in weight from 138 to 124 pounds.

Examination disclosed an intense icterus. The gallbladder was barely palpable, but not tender. The temperature was recorded as 100.6 on the day of admission, but remained normal for the next two weeks. The urine showed a large amount of bile, but no albumin or sugar. The stools were mushy, clay colored, gave a strongly positive Weber test for blood, and contained fat in abundance and some starch. The stomach contents after Ewald's test breakfasts showed no blood and were of normal acidities. Duodenal contents, aspirated by means of an Einhorn tube, contained blood and ferments, but no bile.

A diagnosis of organic disease of the head of the pancreas was made, which was confirmed by operation on Dec. 6, 1915. The patient showed marked

improvement following operation, gaining 12 pounds in weight. A second operation was made on Jan. 22, 1916, and the mass in the head of the pancreas was found to be smaller. Probable diagnosis was made of ulcer of the duodenum with secondary chronic pancreatitis and obstructive jaundice.

An intravenous glucose tolerance test on this patient made Nov. 22, 1915, gave the results shown in Table 5. The weight of patient was 54.3 kg.

TABLE 5.—Glucose Solution, 20.25 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.2	0.89	30	0		
0.5	2.24	70	0		
0.7	3.13	32	Trace	0.1	0.25
0.9	4.02	35	++	0.66	2.09

The threshold of tolerance lies at or very close to 0.7 gm. per kilogram of bodyweight per hour, which is slightly below normal.

Case 6.—Diagnosis, mild diabetes. R. D., a physician, aged 33, showed a trace of sugar in the urine in a recent examination. In one twenty-four hour specimen this had amounted to 0.2 per cent. He had suffered from an attack of appendicitis and underwent an operation in 1911. Two sisters had died of tuberculosis and one brother was obese, weighing 225 pounds.

The patient was a tall, muscular man. The tonsils were small and cryptic. There were a few fillings in the teeth and the gums were retracted at the roots of the left upper molars. The thyroid was not enlarged. A urinalysis was negative.

An intravenous glucose tolerance test was made and resulted as shown in Table 6. The weight of the patient was 83.2 kg.

TABLE 6.—Glucose Solution, 21.94 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.5	3.16	30	++	0.69	0.53
Subsequent loss					1.19+
Total glycosuria					1.72+

The threshold of tolerance lies well below 0.5 gm. per kilogram of bodyweight per hour. The urine was only collected for one hour after the injection stopped.

Case 7.—Diagnosis, mild diabetes. W. K., aged 49, was admitted to the service of Dr. Billings at the Presbyterian Hospital on June 23, 1916. His complaints were aching pains in the right thigh and the suspicion of diabetes.

He gave a history of excellent health up to 1910, when sugar was found in his urine. At that time he was losing weight, had excessive thirst and hunger and polyuria. He was placed on a restricted diet and was soon apparently normal. During the last year he has had rather frequent urination and for the

last three months has suffered from pains in the right thigh and a feeling of weakness in the legs on ascending stairs.

Examination showed a well-nourished man of average height. The teeth were badly discolored and decayed and the Roentgen ray revealed a chronic abscess at the root of one molar. The gums were retracted. A slight systolic murmur was audible at the apex. In all other respects the examination was negative.

Urinalysis of twenty-four-hour specimens showed 1,500 to 1,800 c.c., with specific gravities of 1.010 and 1.013. No reduction of Haines' solution was obtained and there was no evidence of diacetic acid or acetone.

An intravenous d-glucose tolerance test was made on June 26 with results as shown in Table 7. The weight of the patient was 75.4 kg.

TABLE 7.—Glucose Solution, 16.4 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.3	2.29	80	0		
0.5	3.06	80	+	0.5	2.225

The threshold of tolerance lies between 0.3 and 0.5 gm. per kilogram of body weight per hour.

Case 8.—Diagnosis, hyperthyroidism. M. R., a girl, aged 19, complained on admission of goiter, nervousness, dyspnea, palpitation, and edema of the feet. The trouble began as nervousness eighteen months ago. Six months later the thyroid enlarged and the eyes became prominent, and before admission the right lower jaw had often been swollen and painful. Menstruation had been normal.

On examination the patient presented a marked exophthalmos, a fairly large goiter of uniform, firm consistence over which a double murmur could be auscultated, a fine tremor of the tongue and fingers and a pulse ranging from 120 to 140. Adenoids and tonsils were hypertrophic. In the tonsils were deep crypts which contained pus. There were several broken-down teeth at the roots of which abscesses were demonstrable.

Blood counts revealed a moderate anemia and a mononucleosis of from 40 to 46 per cent. Urinary examinations showed an albuminuria of less than 0.1 per cent., a few casts, but no erythrocytes and no sugar.

An intravenous glucose tolerance test was made on this patient with results as shown in Table 8. The weight of the patient was 70 kg.

TABLE 8.—Glucose Solution, 21.8 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.6	3.21	69	+	0.267	1.92
				Subsequent loss	2.12
				Total glycosuria	4.04

The threshold of tolerance lies below 0.6 gm. per kilogram of body weight per hour.

Case 9.—Diagnosis, hyperthyroidism. E. L., a woman, aged 32, was admitted to the service of Dr. B. W. Sippy at the Presbyterian Hospital on Jan. 16, 1916, with complaints of goiter, bulging eyes, palpitating heart, nervousness and weakness. The family history was remarkable in that all the female members of the mother's side, including the grandmother, had large goiters. None had suffered from symptoms of exophthalmic goiter.

The patient had no goiter until the onset of the present trouble, but was always nervous. She had measles at 9, and had suffered from catarrh of the nose and throat from her eighteenth year. At 25 came an acute attack of tonsillitis. Her present illness commenced two months before admission with painful swelling of the thyroid. The pain disappeared in a week, but the swelling persisted; exophthalmos, palpitation and tremors appeared; she grew very nervous and lost weight rapidly.

Examination revealed a young woman in a fair state of nutrition. Exophthalmos was extreme and von Graefe, Möbius and Stellwag signs were present. The goiter was of medium size and of a uniformly firm consistence. A systolic bruit was heard over it. Submanubrial dulness was 7 cm. wide (thymus?). The heart was normal in size, the pulse 112 and regular. The leukocyte count was 8,500 with 45 per cent. mononuclears. The urine was normal on repeated examination.

An intravenous sugar tolerance test was made with results as shown in Table 9. The weight of the patient was 62.3 kg.

TABLE 9.—Glucose Solution, 17.6 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.5	2.95	45	0		
0.6	3.54	46	+	0.5	3.23
Subsequent loss					3.41
Total glycosuria					6.64

Tolerance lies between 0.5 and 0.6 gm. per kilogram per hour.

Case 10.—Diagnosis, hyperthyroidism. A. S., a man, aged 41, was admitted to the service of Dr. C. B. Davis of the Presbyterian Hospital on Aug. 16, 1915, with complaints of goiter, prominence of the eyes, palpitation, nervousness and loss of weight. His disease began in 1903 with thyroid swelling and nervousness. Exophthalmos occurred in 1904 and until 1905 he was very ill. Then followed a period of improvement, lasting until 1913, but after 1913 he suffered from extreme nervousness and palpitation and has lost twenty-five pounds in weight. Sugar has been found in the urine on several occasions.

When examined the patient presented the typical appearance of exophthalmic goiter. He was very thin, very weak and highly nervous. Exophthalmos was extreme, and von Graefe, Möbius and Stellwag signs were positive. The tumor of the neck was large and of a uniformly hard consistence. Over it was heard a systolic, diastolic bruit. The veins of the neck were distended. The heart was enlarged to the left, the apex lying in the fourth space, 11 cm. to the left of the midsternal line. A soft systolic murmur occurred over the apex; a louder systolic murmur was heard at the base and this was transmitted to the neck. The pulse rate was 104. A blood count on the day of admission gave 6,150 whites, with a mononuclear percentage of 29. The urine was normal.

An alimentary test for tolerance, 50 gm. of glucose, was made on Aug. 17, 1915, and 1.6 per cent. glycosuria obtained. Seventeen gm. of sugar were excreted and traces of sugar appeared in the urine for several days.

On Aug. 24, 1915, the upper poles of the thyroid were ligated.

The intravenous tolerance test was made on Feb. 3, 1916, with results as shown in Table 10. The weight of the patient was 53.2 kg.

TABLE 10.—Glucose Solution, 17.67 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.5	2.51	31	0		
0.6	3.01	33	++	0.48	1.73
Subsequent loss					13.39
Total glycosuria					15.12

Tolerance lies between 0.5 and 0.6 gm. per kilogram per hour.

Case 11.—Diagnosis, hyperthyroidism, myositis. J. S., aged 52, was admitted to the Presbyterian Hospital on the service of Dr. Frank Billings with complaints of soreness and weakness of the muscles. The trouble started acutely in May, 1915, with a fever of 104, headache, great muscle soreness and severe weakness. He lost 50 pounds in weight.

Examination revealed extensive muscular atrophies and contractures and tenderness of many tendons. There were no deformities of the joints. In addition, the thyroid was moderately enlarged and there was a fine tremor of the hands and tongue. Ocular symptoms were absent. There was also a diffuse dilatation of the heart and a loud mitral systolic murmur could be heard at the apex. Both great toe nails were ingrown and infected, the tonsils appeared diseased and the teeth were in bad condition. Blood counts gave 3,700,000 erythrocytes, 65 per cent. hemoglobin and 6,300 leukocytes, of which 42 per cent. were mononuclears. Uranalyses were normal. The pulse was fast, ranging from 90 to 120. The temperature proved irregular, occasionally mounting to 101 or 102 F.

TABLE 11.—Glucose Solution, 22.36 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.5	1.75	44	0		
0.6	2.1	41	0		
0.7	2.45	46	+	0.21	0.34
Subsequent loss					1.94
Total glycosuria					1.97

The threshold of tolerance lies below 0.7 gm. per kilogram of body weight per hour.

From October 6 to October 12 the patient received 9 gm. of thyroid extract daily and within forty-eight hours the thyroid became more prominent and the nervousness and tremors increased. The thyroid extract was discontinued. The tonsils were removed and the teeth attended to.

An oral sugar tolerance test was made on October 22. One hundred gm. of glucose given on the fasting stomach produced no glycosuria.

An intravenous glucose tolerance test was made on November 29, and resulted as shown in Table 11. The weight of the patient was 47 kg.

Case 12.—Diagnosis, hyperthyroidism. A. W., a woman, aged 30, suffered from goiter, dyspnea, and palpitation for four years. An attack of tonsillitis with fever immediately preceded the first appearance of these symptoms. Another severe attack of tonsillitis three years later was followed at once by nervousness, tachycardia and loss of weight.

The patient presented a moderate enlargement of the isthmus of the thyroid and a von Graefe. The pulse ranged from 90 to 110. Exophthalmos and tremor were absent. The tonsils were atrophic, but showed in their scarred fibrous surfaces and adhesion to the pillars definite evidence of infection. A moderate anemia was found. The leukocyte count was 8,000 and the mononucleosis 45 per cent. The urine was normal.

An intravenous tolerance test resulted as shown in Table 13. The weight of the patient was 49.54 kg.

TABLE 12.—Glucose Solution, 40.59 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.5	1.02	60	0		
0.7	1.42	30	++	0.43	0.75
Subsequent loss					0.95
Total glycosuria					1.70

The threshold of tolerance lies well below 0.7 gm., but above 0.5 gm. per kilogram of body weight per hour.

Case 13.—Diagnosis, myxedema. Mrs. A. L., aged 64, was admitted to the Presbyterian Hospital on Aug. 15, 1916, with complaints of thickened skin, stiffness of the hands and feet and mental dulness. The family history was negative except for one daughter, who developed an exophthalmic goiter when 40 years of age. The patient has had twelve healthy children, and gives no history of syphilis. She was always well up to her 61st year, when symptoms of the present trouble appeared.

The diagnosis of myxedema was made at the Central Free Dispensary in 1913, and for several months the patient received thyroid extract, but for the last two years she has been away and has had no treatment.

Examination showed the patient to be an elderly Jewish woman who appeared uninterested in her surroundings and mentally dull. The skin was dry and stretched tight over the arms and legs. It had a board-like or bacony feel, especially over the face and arms. There were no axillary hairs, the eyebrows were scanty, and the hair of the scalp was thin and very dry. The pulse was slow, 60 to 70, and the respiration was shallow. The thyroid was not palpable. The lungs had normal resonance, and the breath sounds were clear, except for occasional moist râles at the base. The abdomen was negative.

The temperature was normal. The blood count gave 3,780,000 red cells, 8,100 white and a hemoglobin percentage of 75. The urine was normal in amount and free from albumin or sugar.

The intravenous tolerance test gave results as shown in Table 12. The weight of the patient was 70 kg.

TABLE 13.—Glucose Solution, 18.21 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.8	5.13	48	0		
1	6.41	25	++	0.97	2.14

Subsequent loss not determined.

The second injection rate is well above the patient's tolerance, which undoubtedly lies between 0.8 and 0.9, the normal limits.

Case 14.—Diagnosis, acromegaly. W. F., aged 40, was admitted to the service of Dr. Dean Lewis of the Presbyterian Hospital in 1913, with complaints of blindness and an increase in the growth of the hands, feet and head. His attention was first attracted to his condition in 1905, when he found that his hats were small. A year later his feet outgrew his shoes. In 1907 he was troubled with blurred vision and photophobia. His color sense was lost and finally in 1909 he became totally blind. His weight remained nearly stationary, about 225 pounds, during the entire course of the disease.

Examination revealed conditions typical of acromegaly, hypertrophied bones of the calvarium and jaw, huge hands and enormous feet. The skin was thick and the hair course and dry. An examination of the eyes revealed a complete amblyopia, due to a bilateral optic atrophy. There was also a marked horizontal nystagmus to the left. A Roentgen-ray plate of the skull showed an enormous sella.

The patient remained in the hospital until May, 1915. During this interval the temperature was practically normal and the pulse varied between 80 and 100. Twenty-four-hour urinary volumes were rather high, at times over 3,000 c.c. The urine often contained small amounts of albumin and a few casts, but never any sugar.

TABLE 14.—Glucose Solution, 19.43 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.8	6.91	35	Trace	0.16	0.03
1	8.64	30	++	0.8	0.29
				Subsequent loss	2.01
				Total glycosuria	2.33

The threshold of tolerance lies very close to 0.8 gm. per kilogram of body weight per hour.

An oral glucose tolerance test was made on March 15, 1915. On giving 125 c.c. of glucose at 7 a. m., no glycosuria resulted.

The patient returned to the hospital in November. His condition had remained practically stationary. An intravenous tolerance test was made and resulted as shown in Table 14. The weight of the patient was 103.6 kg.

Case 15.—Diagnosis, acromegaly. C. H., man aged 38 was admitted to the service of Dr. Dean Lewis of the Presbyterian Hospital on Jan. 4, 1916, with complaints of diminishing vision, increasing size, headache, weakness and loss of sexual desire. The patient was an adopted child and could tell nothing about his parents. At 7 years of age he had a severe illness the nature of which he ignores, and in 1906 suffered from a light attack of malaria. He was married in 1915 and had no children. The present illness seems to have begun in 1913, when he began to increase in size and weight. He is now 6 feet 1 inch tall and weighs 210 pounds. His former weight was 185. His feet and hands are bigger and he is obliged to buy 7¼ inch size of hats instead of his former size 6⅞. Visual disturbance first occurred in February, 1915.

On examination the findings were those typical of acromegaly and roentgenograms of the hypophysial region revealed a sella very much enlarged. The leukocyte count was 7,600, with 58 per cent. mononuclears. The urine was normal on repeated examination. Two hundred gm. of glucose were given by mouth without producing glycosuria.

An intravenous tolerance test resulted as shown in Table 15. The weight of the patient was 95.5 kg.

TABLE 15.—Glucose Solution, 19.01 Per Cent.

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.7	5.85	30	0		
0.9	7.52	30	++	3.18	2.7
Subsequent loss					7.98
Total glycosuria					10.68

Tolerance lies below 0.9 gm., but above 0.7 gm. per kilogram per hour.

Case 16.—Diagnosis, hypophysial disease (Frölich syndrome). H. C., a girl, aged 14, complained of obesity, delayed sexual development and frontal headache. The headaches began when she was about 10 years old. At 12 obesity was noticeable. At 13 she became uncertain of her balance and would sometimes fall to the ground, without, however, losing consciousness. She has never menstruated.

Examination revealed a corpulent child of small stature, mentally very bright. The head was larger than normal. The vision was good and the color fields were unaltered. The pupils, however, reacted sluggishly and both optic disks were abnormally pale, so that an early optic atrophy was diagnosed. Secondary sexual characteristics were lacking.

Urine examinations were completely negative and the twenty-four-hour urinary amounts were normal. The spinal fluid contained only 5 cells per c.mm. and gave negative Wassermann, Nonne and Lange tests. Roentgen-ray plates of the head showed a sella much increased in size. A subsequent decompression operation revealed marked intracranial pressure.

An intravenous glucose tolerance test was made on this patient before the decompression operation was performed and resulted as shown in Table 16. The weight of the patient was 47 kg.

TABLE 16.—Glucose Solution, 19.43 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.8	3.23	42	+	0.31	0.372
Subsequent loss					0.405
Total glycosuria					0.777

The threshold of tolerance lies a little below 0.8 gm. per kilogram of body weight per hour. There is certainly no increase over normal.

Case 17.—Diagnosis, hypophysial disease (gigantism?). T. W., a man, aged 26, was admitted to the service of Dr. D. P. Abbott at the Presbyterian Hospital on Jan. 10, 1916, with complaints of temporal headaches, weakness and nervousness. The headaches were of only a few days' duration. The weakness was not great. The nervousness had been present since childhood. The patient had no severe illnesses other than a chronic infection of the right ear which began early in childhood and only healed in 1912. He was, however, slow in learning to walk (2 years), was always big for his age and had always been backward in school. His family history was negative and venereal infection was denied.

Examination revealed a man 6 feet 1 inch tall, and big in proportion. The sexual development, pubic hair, etc., appeared normal. There was an old perforation in the right ear drum. A roentgenogram of the hypophysial region showed an unusually small sella and it was thought probable that a correlation existed between this and the rather peculiar development.

An intravenous glucose tolerance test resulted as shown in Table 17. The weight of the patient was 80.7 kg.

TABLE 17.—Glucose Solution, 19.2 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.2	1.4	30	0		
0.7	4.9	30	0		
0.8	5.6	30	++	.....	Not determined

Tolerance lies between 0.7 and 0.8 gm. per kilogram per hour. It may be subnormal, but is certainly not increased.

Blood sugar determinations were made before and after the injections by Dr. G. Pearce and showed a percentage of 0.115 and 0.212, respectively.

Case 18.—Diagnosis, hypophysial disease, diabetes insipidus. M. L., a man, aged 40, was admitted on the service of Dr. Joseph Miller at the Cook County Hospital in August, 1915. His complaints were polyuria and sexual impotence.

The first symptoms of disease occurred in 1907 and consisted of severe headache, occasional vomiting and excessive thirst. He would drink from 10 to 12 gallons of water a day. Sexual power was lost in 1911. Since 1913 he has been free from headaches and the thirst has been somewhat less. The appetite has always been moderate. He had four or five gonorrheal infections, and in 1901 a genital chancre. He was treated for this and no secondary symptoms of syphilis followed.

Examination revealed a moderately corpulent man of medium height and with small bones. The pubic hairs were found scanty and had a feminine distribution. The beard was very thin. Penis and testicles were small. In the right eye was a cataract, in the left the vision was good, but the red and green color fields showed some interlacing of their margins. The urine was found to be large in amount, the twenty-four-hour specimens containing from 3,000 to 6,000 c.c. The specific gravity was low and sugar was absent. Roentgenograms of the head revealed a slight enlargement of the sella and destruction of the posterior clinoid processes.

The intravenous glucose tolerance test resulted as shown in Table 18. The weight of the patient was 63.6 kg.

TABLE 18.—Glucose Solution, 20.11 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose		
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope	Total Gm.
0.5	2.64	43	0		
0.6	3.16	59	0		
0.8	4.22	60	0		
0.9	4.75	37	+	0.29	0.59
Subsequent loss					0.86
Total glycosuria					1.45

The threshold of tolerance lies between 0.8 and 0.9 gm. per kilogram of body weight per hour.

Case 19.—Diagnosis, cirrhosis of the liver. G. R., a man, aged 47, was admitted to the service of Dr. Billings at the Presbyterian Hospital on June 29, 1916. His complaints were loss of weight, weakness and pigmentation of the skin.

The history contained a record of scarlet fever at the age of 11 and malaria at 25, but excepting these illnesses, good health up to the spring of 1916.

Since April he has felt weak, has decreased in weight from 202 to 162 pounds, and has observed a pigmentation of the skin of the entire body. There has never been any icterus of the sclera and the color of the skin is more brown than yellow. In May he visited the dispensary, and from there was sent to the hospital. His family and venereal records are negative, but for years he has been drinking daily very considerable quantities of beer and whisky.

Examination revealed a poorly nourished man of average height. The pigmentation of the skin was unequally distributed, the nipples, pubic region and axillae were very dark; there were no spots in the mouth and the sclera were clear. There was a moderate pyorrhea and the teeth were discolored. The chest was normal in shape, the lungs slightly emphysematous, the heart borders somewhat wider than normal. A slight systolic blow was heard at the apex. The abdomen was somewhat pendulous, but contained no fluid. The spleen was palpable during inspiration, very firm, smooth and not tender.

The upper border of the liver was found at the fourth rib; the lower border extended 6 cm. below the costal arch in the mammary line. The edge was firm and regular and the surface was smooth

The blood pressure, systolic, was 140, diastolic 70. Wassermann tests were negative. A blood count gave 3,500,000 erythrocytes, 4,000 leukocytes and 70 per cent. hemoglobin.

The urine was examined repeatedly without finding sugar or bile pigments. The stools contained bile in normal amount.

On July 2, 50 gm. of glucose were given on the empty stomach without leading to melituria. Levulose and galactose tolerance tests were omitted, owing to the impossibility of obtaining these sugars.

An intravenous d-glucose tolerance estimation, made on July 5, 1916, resulted as shown in Table 19. The weight of the patient was 74 kg.

TABLE 19.—Glucose Solution, 17.48 Per Cent. by Polariscope

Rate of Injection		Minutes of Injection	Urine Glucose	
Gm. per Kg. per Hr.	C.c. Solution per Min.		Haines' Test	Per Cent. by Polariscope
0.4	2.82	40	0	
0.6	4.23	20	0	
0.8	5.64	15	0	0

Tolerance is above 0.8 gm. per kilogram of body weight per hour and is apparently normal.

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STUDIES ON THE THEORY OF DIABETES.

VIII. TIMED INTRAVENOUS INJECTIONS OF GLUCOSE AT LOWER RATES.*

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INTRODUCTION.

Principles underlying the method of timed intravenous injections at known rates, and an improved pump designed for the purpose were recently described.[1] Their application in a clinical study of glucose tolerance has been reported elsewhere by Wilder and Sansum.[2] This paper deals generally with the technique of the experiments carried out on rabbits and dogs, and, in particular, with observations concerning the effects of sustained intravenous injections of glucose at rates up to 2 gm. per kg. per hour. The experiments with higher rates of injection will be made the subject of another paper. Apart from recording certain data concerning the behavior of glucose and water, the purpose of this paper is further to discuss the relative importance of rate and concentration measurements in biological work generally.

Methods.

Material.—The pure glucose used in the majority of experiments was prepared in the laboratory as described below from a completely hydrolyzed granular commercial glucose from cornstarch.[3] A Kahlbaum c. p. glucose preparation was used at in-

* A third detailed report on phases of the work outlined by Woodyatt, R. T., Sansum, W. D., and Wilder, R. M., *J. Am. Med. Assn.*, 1915, lxv, 2067.

[1] Woodyatt, *J. Biol. Chem.*, 1917, xxix, 355.

[2] Wilder and Sansum, *Arch. Int. Med.*, 1917, xix, 311.

[3] Made at the Argo plant of The Corn Products Refining Company

tervals for control purposes. Some lots of the product made in the laboratory caused melituria when injected at rates below that found necessary with the Kahlbaum preparation.

1,000 gm. of the crude glucose are dissolved in 400 cc. of boiling water digested 1 to 2 hours (not longer) with animal charcoal and the solution filtered hot by suction. Add 800 cc. of 95 per cent alcohol, cool, and inoculate with glucose crystals. The first crystals (5 to 10 gm.) carry down suspended carbon and are filtered off. As further crystallization proceeds, filter off successive fractions to avoid caking. Wash the crystals with cold alcohol on a suction funnel. (Save the alcoholic washings to dilute the next concentrated crude glucose filtrate.) Yield = 50 to 60 per cent. Repeat the process if necessary. If the crude glucose employed contains much tarry matter, it may be necessary to use more than 400 cc. of water per 1,000 gm. at the outset to obtain a filterable solution. The final pure white product was made up into 1, 2, 3, and 4 molecular (18, 36, 54, or 72 per cent) solutions with water freshly distilled as for a salvarsan injection and placed in 2 to 3 liter flasks in which they showed only a straw or light yellow color. This color may be wholly removed by a final clearing with a little bone-black. After sterilization and before use, the exact strength of each solution was determined by polariscope checked at times by a reduction method. Solutions of 1 and 2 molecular strength proved most generally useful.

It may be noted that a 1 gm. molecular or 18 per cent aqueous glucose solution contains, at 17.5°C. per 100 cc. volume, 89.44 gm. of water (Baling) and 18 gm. of glucose, or 1 molecule of glucose to 50 molecules of water, whereas a 2 gm. molecular (36 per cent) aqueous glucose solution contains, per 100 cc., 79.90 gm. of water and 36 gm. of glucose, or 1 molecule of glucose to about 22 molecules of water.

The subjects were dogs, rabbits, and human beings. The bulk of the work was with dogs weighing from 9 to 19 kg. Care was taken to select vigorous animals of gentle and phlegmatic dispositions received from active outdoor life. Bull terriers or mongrels with a strong admixture of bulldog blood were used in most of the satisfactory experiments, whereas most of the failures occurred with unhealthy, housed, ill fed, or nervous animals.

Technique.—The technique of the experiments as applied to human subjects was the same as that described and illustrated by Wilder and Sansum[2] in their report on clinical glucose tolerance determinations by the intravenous method. The routine of the dog experiments requires further description.

The solution to be injected is poured into a previously sterilized burette of about 500 cc. capacity. The burette has a glass cock and the tip connects by rubber tubing with one arm of a glass Y-tube from the stem of

which rubber tubing leads to the suction nipple of a volumetric pump. A second slender burette beside the first connects with the other arm of the Y-tube and serves for short interval readings. Half hourly or hourly rates are checked on the reservoir burette. One or two coils of the rubber tubing which carries the solution from the burettes to the pump are allowed to rest in a water bath to warm the sugar solution. The delivery tube from the pump terminates in a small cannula inserted into a vein, usually at the ankle in the case of dogs. With rabbits the injections have been through a small needle inserted into the ear vein and held by a small clamp. In many of the experiments two pumps have been employed, the second or auxiliary taking distilled water from a second pair of burettes and discharging it through a delivery tube and a needle which is thrust obliquely through the wall of the rubber delivery tube of the main pump; or, the two delivery tubes may be permanently anastomosed. Thus it is made possible *to control the concentrations of water and glucose as well as the absolute quantities in which they are injected.*

The dogs lie on their backs or partly on the side in an ordinary animal trough cushioned with blankets. There is a leather collar about the neck and a not too snug jacket or flannel bandage on the chest. Collar and jacket are secured below to the animal board allowing limited freedom of movement, care being taken not to impede respiration. The fore legs are free. The hind legs are separated to the opposite lower borders of the board and held firmly by slings but not in such a way as to cause pain or stop circulation.

A catheter is placed in the bladder and secured by a strip of adhesive tape fixed to the catheter and carried upward on to the abdomen. It was found desirable to accustom the dogs to these procedures in sham experiments on previous days before attempting long injections. Preferably, exposure of the vein has been carried out under novocaine on the day before the experiment, in which case ligatures have been laid, the wound smeared with vaseline, and the whole enclosed with a band of adhesive tape. In the experiment the cannula may be inserted without causing pain. These precautions were taken to avoid non-physiological factors and because experience proved that restlessness, discomfort, pain, or emotion could affect the results, especially the rate of water secretion. In many experiments the laden animal board has been made to rest on the platform of a scales and the scale beam so balanced that gains or losses of body weight incident to the development of positive or negative water balances could be detected and, if desired, corrected by changing the rate of the water injection by means of the auxiliary pump.

The animal experiments have all been conducted in a separate room from which noise and confusion could be excluded. In satisfactory experiments dogs have lain quietly for many hours at a time, the pumps have required little attention, and the collection of urine has proceeded automatically except for required manipulation at the end of the hourly periods.

EXPERIMENTAL.

As an aqueous solution of glucose may be regarded as representing merely so many molecules of glucose, so many of water, or as glucose and water together, each affecting and affected by the other in a way not fully understood, to form among other things a series of hydrates and their dissociation fragments in accordance with the solvate theory,[4] so intravenous injections of aqueous glucose solutions and their physiological effects have to be considered from at least these viewpoints. Although in this report attention is centered on the glucose this necessarily involves some consideration of the corresponding movements of water into and through the body.

Continued Injections of Glucose into the Systemic Blood at Rates Not Greater Than 1.0 Gm. per Kilo of Body Weight per Hour.

In all, upwards of fifty experiments have been conducted in man, dogs, and rabbits, receiving glucose by vein at the lower rates. Conditions were considered standard when the subjects were healthy, well nourished, mature individuals in a state of complete bodily rest and free of pain, discomfort, emotion, or other tangible nerve-disturbing influence, when the glucose employed reacted chemically and physiologically as pure *d*-glucose and when the solutions employed varied in strength between 16 and 40 per cent. The gm. molecular (18 per cent) solution was most commonly used. The duration of the experiments varied from ½ hour to 7 hours.

In man, injections at the rate of 0.8 gm. per kg. per hour were not observed to produce glycosuria detectable by the ordinary test solutions employed (Fehling's, Haines'), after 30 minutes to 3 hours of injection. *In dogs and rabbits* the same results were obtained. One dog received glucose at this rate for 7 hours with the same result. Injection at the rate of 0.9 gm. per kg. per hour *in man* uniformly caused glycosuria, also *in rabbits and dogs* in the great majority of cases but not in all. In four dogs it required 1.0 gm. of glucose per kg. per hour to cause glycosuria. It

[4] Cf Jones, H. C., Absorption spectra of solutions as studied by means of the radio-micrometer, *Carnegie Institution of Washington, Publication No. 210*. 1915. A summary of earlier literatures is given at the end.

has been observed that glycosuria has failed to develop when the injection rate has been between 0.8 and 0.85 gm., and appeared when the rate has been advanced to between 0.85 and 0.90 gm. per kg. per hour.

The time elapsing between the beginning of an injection at the 0.9 gm. rate and the detection of glycosuria was always short when this rate was instituted directly after ½ hour or more of injection at the rate of 0.6 to 0.8 gm. per kg. per hour, and longer when the 0.9 gm. rate was instituted with no prior glucose administration. Owing to the time necessary for enough urine to collect in the bladder, to remove and test satisfactorily, the exact interval elapsing between the beginning of the 0.9 gm. rate and the actual secretion of saccharine urine by the kidneys cannot be stated, but it was found to be as long as 40 minutes with one dog which had received no prior administration, and as short as 6 minutes in a dog which had received glucose for ½ hour at the 0.8 gm. rate before the rate was advanced. Following preliminary injections at a subtolerant rate for ½ hour or more, an advance to the 0.9 rate has regularly caused sugar to appear in the urine within the first half hour.

Conclusions.—On the basis of all the experiments taken collectively it is reckoned that the maximum rate at which glucose can be administered into the systemic blood and still fail to produce glycosuria in normal resting human individuals is close to 0.85 gm. per kg. per hour. Perhaps in dogs and rabbits it averages a little higher than in man. (These statements agree in the main with those made in our preliminary report on the basis of experiments then completed. They take into account the later series of observations on human subjects described by Wilder and Sansum, and further dog and rabbit observations which permit the correction of slight inaccuracies in the earlier report.)

The Behavior of Water in Conjunction with Sustained Intrasystemic Glucose Injections at Subtolerant Rates.

Two things may be noted here: (1) The same results as described above have been obtained with glucose solutions varying in concentration between 16 and 40 per cent. The method em-

ployed did not permit the detection of any difference of the intravenous glucose tolerance limit as a result of variations between rather wide limits of the quantity of water which accompanied the glucose into the vein. Thus in dogs weighing 10 kg., the limit was found at 0.85 gm. per kg. per hour both with 47 cc. of 1 M (18 per cent) and with 23.5 cc. of 2 M (36 per cent) glucose solutions. Even when solutions containing 72 to 100 gm. of glucose per 100 cc. have been used, the tolerance limit has not varied by degrees which could be detected by the methods employed. The same has held for solutions as weak as 9 per cent. However, the more concentrated solutions increase the difficulties of maintaining exact injection rates when dealing with dogs and rabbits, and following an injection of 72 per cent solution in one human case, a thrombus developed in the vein so that no further use was made of such strong solutions in the clinic. Too dilute solutions, on the other hand, may introduce such factors as hemolysis and significant changes of weight. (2) During intravenous glucose injections at subtolerant rates there has been observed a strong tendency toward retention of water in the organism, which under appropriate experimental conditions may be manifested by an absolute decrease in the rate of diuresis that appears after the injection has been continued for a sufficient length of time, lasts during the remainder of the injection, and promptly gives way to a sudden increase of diuresis when the injection is stopped. The following experiment is typical.

EXPERIMENT I.

The Retardation of Diuresis during Intravenous Injection of Glucose at a Subtolerant Rate with High Diuresis after Injection.

A dog weighing 12.7 kg. received 40 cc. of water *per os* at 8 and again at 9 a.m. when it was placed on the board, catheterized, and prepared for intravenous injection. Thereafter the bladder was emptied by catheter every hour. The injection consisted of 51.5 cc. of 19.7 per cent glucose solution (containing about 45 cc. of water) per hour. Rate, 0.8 gm. glucose per kg. per hour. The table shows the amounts of urine obtained at the end of each succeeding hour, the bold-faced figures indicating hours of injection.

Hour.	Urine.	Remarks.
	cc.	
10 a.m.	40	
11 "	30	
12 "	**13**	Injection 0.8 gm. per kg.
1 p.m.	**18**	" 0.8 " " "
2 "	**7**	" 0.8 " " "
3 "	**8**	" 0.8 " " "
4 "	**9**	" 0.8 " " "
5 "	**10**	" 0.8 " " "
6 "	**12**	" 0.8 " " "
7 "	80	

Of the 80 cc. of urine passed between 6 and 7 o'clock, 58 cc. appeared in the first 25 minutes. Thereafter for 12 hours the average urinary volume was 12 cc. per hour. It may be noted that the intake of water per hour during injection was about that given in the fore-periods. There was no glycosuria.

We have failed to observe such absolute retardations of diuresis in experiments in which the quantity of water given during the preparatory periods or during the glucose injection periods has been too great. In such cases, however, there has never been noted any increase of diuresis attributable to the glucose itself; *i.e.*, the injection of glucose solutions at subtolerant rates has not been observed to elevate the diuresis more than the injection of equivalent volumes of water in the form of 0.6 to 0.8 per cent sodium chloride solutions. It would appear that in the presence of an excess of water in the organism, the binding of some by the glucose may fail to lessen the absolute rate of diuresis. Such a negative effect is illustrated by the following experiment.

EXPERIMENT II.

The Absence of Retardation of Diuresis during Intravenous Injection of Glucose at Low Rates.

The weight of the dog was 14.3 kg. At 8, 9, 10, and 11 a.m. it received by stomach tube 70 cc. of water (total 280 cc.). At 1 p.m. the bladder was emptied by catheter and thereafter every hour. Injection was begun at 2 p.m. In the 1st hour the rate was 0.8, in the second 0.9, in the third 1.0 gm. per kg., glycosuria appearing 10 minutes after beginning the latter rate. The bold-faced figures show hours of injection. The rate of water supply was the same during the preparatory and the injection hours.

Hour.	Urine.	Remarks.
	cc.	
2 p.m.	44	
3 "	39	Injection 0.8 gm. glucose per kg.
4 "	48	" 0.9 " " " "
5 "	52*	" 1.09 " " " "
6 "	48	

* Glycosuria in the 3rd hour.

Both types of result have been observed during intravenous glucose injections in man, but owing to the undesirability of catheterizing normal individuals for a sharp separation of the urine at short intervals, the greater frequency of psychic and nervous influences, and other factors which make it difficult to secure uniform rates of diuresis during the control periods, observations of diuresis in man have been unsatisfactory. (Although Pavy[5] had described retardation of diuresis during low rate intravenous injections of glucose, we had not succeeded in confirming his observation at the time of our preliminary report.)

Injections at Rates between 1 and 2 Gm. of Glucose per Kg. per Hour.

With injections of 1.8 to 2.0 gm. per kg. per hour glycosuria begins promptly and quickly attains a maximum, after which it proceeds at a uniform rate hour after hour for at least 8 hours and probably much longer. Reference to the record of Experiment III, which is typical, will show that the total excretion during the 1st hour may equal that of later hours, indicating the promptness with which the maximum glycosuria may be established with an injection at this low rate.[6]

The absolute rate of the glycosuria may differ in different individuals, and probably in the same individual under different physiological conditions.

In Experiment III the average excretion was 0.42 gm. per kg. per hour, or 2.1 per cent of the intake. Excretions representing

[5] Pavy, F. W., *J. Physiol.*, 1899, xxiv, 479.

[6] With higher rates of injection (2.7 to 7.2 gm. per kg. per hour) the glycosuria gradually accelerates during the first 1 to 5 hours or more before constancy is finally attained, the time required depending largely on the rates of glucose administration and on the water balance.

about 2 per cent of the intake have been seen in other experiments, but one dog receiving 1.8 gm. of glucose per kg. per hour excreted 1.90, 1.75, 2.04, 1.62, 1.61, and 1.97 gm. of glucose per 10 kg. in successive hours, an average of 1.8 gm. per hour representing 10 per cent of the injection.

The glycosuria has been observed to cease within 6 minutes after stopping the injection; *i.e.*, if the bladder is washed out immediately after ending the injection, the next urine collected has been found free of sugar.

The Behavior of Water during Injections at Rates of 1.8 to 2.0 Gm. per Kg. per Hour.

1. Within rather wide limits the quantity of water given before an experiment or during the glucose injection may be varied without influencing the rate of glucose excretion. This is typically illustrated in the following experiment.

EXPERIMENT III.

The Constant Supply and Constant Excretion of Glucose While the Supply and Excretion of Water Varies.

The subject was a bull terrier weighing 16.7 kg., which had served in four previous experiments of similar character. Two pumps, with the delivery tubes anastomosed, discharged water and 37.01 per cent glucose solution respectively through a single cannula. The pump discharging glucose solution ran at a constant rate to deliver 53.9 cc. per 10 kg. of body weight per hour (containing 20 gm. of glucose and 42.6 cc. of water). The second pump delivered water at rates which were varied between 53.9 and 107.8 cc. per kg. per hour. The rates of injection and elimination of glucose and water per 10 kg. of body weight are tabulated by hours from the beginning of the injection.

Injected.			Excreted.		
Hour.	Glucose.	Water.	Glucose.	Urine.	Urinary glucose concentration.
	gm.	*cc.*	*gm.*	*cc.*	*per cent*
1	20	96.5	0.43	16.8	2.57
2	20	96.5	0.31*	6.2	4.90
3	20	151.5	0.52*	59.8	0.87
4	20	123.5	0.44	118.3	0.37
5	20	96.5	0.42	128.0	0.35
6	20	96.5	0.44	82.5	0.59
7	20	96.5	0.41	58.5	0.62
8	20	96.5	0.42	67.0	0.58

* Average 0.42 gm.

In this experiment it will be noted that in the 2nd hour when the urine was only 6.2 cc. and contained nearly 5 per cent of glucose, the excretion was 0.1 gm. below the average of the 1st hour and the last 5 hours, whereas in the 3rd hour when the urine rose to 58.9 cc. it was a trifle above. But the average of the 2nd and 3rd hours was 0.42 gm., the same as for the others, and this fluctuation would be accounted for if only a 2 cc. residuum of the concentrated urine had remained in the bladder, urinary passages, and kidneys to be flushed out during the diuresis of the following hour. There might of course be some retention in the tissues also if the supply of water was too small. In the 7th hour, with the same urinary volume as in the 3rd, the excretion of glucose was 0.41 gm. These observations parallel those made with injections at the 0.8 to 0.9 gm. rates and imply that the glucose utilization rate, as well as the excretion rate, may remain the same during marked fluctuations of the water balance.[7]

2. Unlike injections at subtolerant rates, those productive of glycosuria, may, as is well known, cause acceleration of diuresis. But as in the case of the former, the more rapid injections may be attended by no absolute change of the rate of diuresis, or they may lead to absolute retardations. Which effect will occur appears to depend wholly on the supply of available water in the organism and on the rate of the glycosuria, other factors remaining constant. In experiments with injections at these rates it is possible, under suitable conditions, to demonstrate diminished diuresis during the injection of 1.8 to 2.0 gm. of glucose per kg., followed by sudden liberation of water into the urine when the injection is stopped, exactly as in the case of subtolerant injections; only to do so the water balance must be kept at a relatively lower level.

Frequently in the course of clinical work with 18 per cent glu-

[7] With higher rates of glucose injection, and with wider variation of the water balance at the present rates, the excretion may be affected. However, these observations would not be in harmony with A. A. Epstein's statement (Studies on Hyperglycemia in Relation to Glycosuria, New York, 1916, 69) that "diuresis in diabetes plays an important rôle in determining the total amount of sugar eliminated in the urine, but has no influence on its concentration." They agree, on the other hand, with the finding of T. Addis and C. K. Watanabe (*J. Biol. Chem.*, 1917, xxix, 404) that "changes in the volume of the urine or in the urea concentration of the urine have no appreciable effect on the rate of urea excretion."

cose solutions, an overstepping of the tolerance has been attended by increased diuresis. With the same solution in dogs, decreased diuresis has at times been observed even when a considerable glycosuria has been in progress, as illustrated in the 2nd hour of Experiment III, but the effect is better shown by experiments in which solutions of 50 per cent concentration or above have been used, as in the following.

EXPERIMENT IV.

Retardation of Diuresis during Intravenous Injection at a Rate Productive of Glycosuria, with Acceleration of Diuresis after Injection.

The dog weighed 17 kg. During the 12 hours preceding the experiment it received no food, but water was provided in the cage. At 8.30 a.m. 55 cc. of water were given by stomach tube and the dog was placed on the animal board and prepared for intravenous injection. At 9.30 it was catheterized and thereafter the urine was drawn by catheter every hour. Intravenous injection was begun with 53 per cent solution at the rate of 1.7 gm. of glucose per kg. per hour; *i.e.*, 55.1 cc. of the solution per hour. The injection was continued for the next 4 hours, ending at 4.30 p.m. At 1.30 the urine contained sugar. At 4.30 it contained 4.4 per cent (total 0.35 gm., representing 2 per cent of the intake).

Hour.	Urine volume.	Remarks.
	cc.	
10.30	32	
11.30	15	
12.30	15	Injection started.
1.30	10	
2.30	8	
3.30	13	
4.30	8	Injection ended.
5.30	65	
6.30	98	
7.30	20	

During the following 12 hours the average urination was 18 cc. per hour.

In this experiment the retardation of diuresis during the injection is comparable to that seen with injections at subtolerant rates. The high diuresis following the discontinuance of injection is even more pronounced and of longer duration.

The highest concentration of glucose which has been observed in the urine during the present experiments with injections at rates below 2 gm. per kg. per hour has been 8.5 per cent.

It may be stated in anticipation of later papers that the urinary glucose concentration, even with injections at rates as high as 7.2 gm. per kg. per hour, has not been driven higher than 8 to 9 per cent except in the case of a few animals. Since in pancreatic and phlorhizin diabetes concentrations of 12 to 13 per cent are easily produced, the point may have theoretical significance.

DISCUSSION.

The Maximum Rate at Which Glucose Can Be Administered Continuously via the Systemic Blood Stream and Fail to Cause Glycosuria.

The value of 0.85 gm. per kg. per hour agrees only approximately with the findings of Blumenthal[8] and others who have used his method (Comessatti,[9] and Loeb and Stadler[10]). Blumenthal made repeated single injections into rabbits with a hand syringe at 15 minute intervals, each injection consuming 10 to 15 seconds during total periods as long as 3 hours. The tolerance limit in dogs (originally expressed in gm. per kg. per $\frac{1}{4}$ hour, and here recalculated for the hour basis) was found as low as 0.6 and as high as 1.3 gm. per kg. The present experiments lasting as long as 7 hours with consistent results varying with few exceptions only between 0.8 and 0.9 gm. define the normal limit more sharply.

The Significance of the Observation That, within Limits, the Quantity of Water Administered before or during Sustained Glucose Injections at Fixed Rates Need Not Affect the Rate of Glucose Utilization or Elimination.

Whatever variations of the blood and tissue volumes and of the blood and urinary sugar concentrations were involved in the fluctuations of the water balance, they did not noticeably affect

[8] Blumenthal, F., *Beitr. chem. Phys. u. Path.*, 1905, vi, 329.

[9] Comessatti, G., *Beitr. chem. Phys. u. Path.*, 1907, ix, 67.

[10] Loeb, O., and Stadler, H., *Arch. exp. Path. u. Pharm.*, 1914, lxxvii, 326.

the rate of glucose metabolism or excretion as long as the rate of glucose supply was constant. Epstein,[11] working with diabetics, also found that the quantity of glucose excreted might be the same in cases showing different blood sugar concentrations and *vice versa*. Such an effect is strikingly illustrated by an unpublished experiment of our own in which a dog received glucose by vein at the rate of 36 gm., and then 27 gm. per 10 kg. per hour, while the water administration was voluntarily varied with a second pump. In the last hour of the faster glucose injection the excretion was 15.2 gm.; in the 1st hour of the reduced rate of injection, it was 10.23 gm., but the blood sugar concentration in both hours was 0.89 per cent. The occurrence of such phenomena is of course tacitly implied in Ambard's attempts to formulate laws governing them. They are also in keeping with the older observation that the drinking of water and passage of urine need not affect the protein metabolism or the total heat production of the organism, as illustrated specifically by a calorimeter experiment of Lusk's[12] in which an 8.8 kg. dog received 200 cc. of water by stomach with no influence on the metabolism. *But the question arises as to how water may dispose itself in the organism to permit such results and how their recognition bears on current conceptions of the significance of measurements of the concentration of various substances in the blood.*

It is clear that the rate of supply to a given organism at a given time is the chief factor which determines the velocity of chemical change and the rate of elimination, but the mechanism is less obvious. Variations of the total quantity of a glucose solution of fixed concentration on one side of an ordinary dialyzer would not vary the rate at which glucose molecules diffused through it, nor could the concentration be changed *without* affecting the rate of diffusion. But with the kidney membrane and cells in general it is not merely the concentration which determines the rates of excretion and utilization respectively. This would suggest that the surfaces of contact between the plasma and the cells must vary in a definite manner with the volume and glucose concentration of the plasma.

[11] Epstein;[7] see also *Proc. Soc. Exp. Biol. and Med.*, 1916, xiii, 67, and Epstein, A. A., and Baehr, G., *J. Biol. Chem.*, 1914, xviii, 21

[12] Lusk, G., *J. Biol. Chem.*, 1912–13, xiii 41 and 45.

The following, in which cells and plasma represent phases of a system, serves for illustration. If a single spherical cell such as a lymphocyte is suspended in plasma containing glucose, then (when a state of equilibrium has been approached) the velocity at which glucose molecules strike and penetrate its surface, and therefore the velocity at which they react chemically with substances in the cell interior, will, other factors remaining the same, be determined by the concentration of glucose in the plasma and the area of the surface of contact between the cell and the plasma. If the concentration of glucose in the plasma were decreased, by increasing the volume through the addition of water, and *if the cell surface remained unchanged*, this would lessen the frequency with which glucose molecules impinged on the cell, and entail a proportional decrease of the number entering in a unit of time, etc. In like manner, abstraction of water from the plasma with a decrease of its volume and increase of the glucose concentration would increase the rate at which glucose molecules entered the cells and reacted within it. In either of these hypothetical cases the volume change in the plasma would involve changes in the rate of the glucose metabolism of the cell, contrary to the experimental findings under discussion. If, however, as the plasma volume increased or diminished (and the glucose concentration varied correspondingly) the surface of contact between the cell and the plasma increased or diminished in the same proportion, then the total number of glucose molecules impinging on the total cell surface would theoretically remain the same and such an occurrence would explain the experimental findings.

The necessary condition would be fulfilled if while the total content of glucose in the plasma remained fixed and the volume of plasma varied, the ratio of the plasma volume to the surface of contact between the plasma and the cell remained constant; that is, in the body taken as a whole, if *the surface of contact between the plasma and cell phases varied directly as the plasma volume or inversely as the plasma glucose concentration.*

These observations are in harmony with those of Epstein concerning the ratio of the plasma volume to the blood cell volume, the blood sugar concentration, and the rate of glycosuria in diabetics, which led to the conclusion that the total sugar content of the blood fixes the rate of glycosuria. Other things being equal,

it should do so in accordance with the above, *as long as the specific surface of the plasma remains fixed.*

As to how the surface of contact between the plasma and the cells of the body may be regulated to meet changing volumes of blood, several ways are apparent. In the histological sense, the single leukocyte suspended in plasma may increase in volume as water is added to the plasma, or diminish when glucose is added. Dilution of the plasma may separate cells in contact, concentration permit separate cells to cluster together. The wall of the capillary may be more or less distended, the glomerular tuft may inflate and deflate, providing more or less surface in proportion to the blood volume. Or the question may be considered in terms of phases in colloid systems, and the influence of varying contents of sugar and water on their volumes and specific surfaces. As Henderson[13] recalls, Willard Gibbs proved that when equilibrium is established the state of the system is unaffected by the volume of the phases.

Acceleration and Retardation of Diuresis Resulting from Intravenous Injections of Glucose.

The phenomenon of decreased diuresis during injection, with a sudden liberation of water into the urine when the injection is ended, is quite analogous to that observed as a result of alimentary glucose administration by Lusk[12] and Fisher and Wishart.[14] The analogy is made striking by a recalculation of their data, which shows that glucose actually entered the blood by absorption during periods of 2 to 5 hours at mean rates of 1.6 to 1.7 gm. per kg. per hour, as compared with direct injections of 0.8, 1.8, and 2.0 gm. per kg. per hour in the present series.

Fisher and Wishart administered 50 gm. doses of glucose by stomach to dogs weighing 8 to 9 kg., sacrificed them at hour intervals, and found that absorption was completed "during the 4th hour." If the absorption time was 3.5 hours and the average body weight 8.5 kg., this would imply a mean absorption rate of 1.68 gm. per kg. per hour. Lusk found that 50 gm. of glucose might cause a 20 per cent increase in metabolism ending "*in the 4th hour*," and 75 gm. might also cause a 20 per cent increase

[13] Henderson, L. J., *Proc. Nat. Acad. Sc.*, 1916, ii, 654.

[14] Fisher, G., and Wishart, M. B., *J. Biol. Chem.*, 1912–13, xiii, 49.

which continued "*through the 5th hour.*" As Lusk states, Fisher and Wishart showed "that there is a large retention of water by the organism during the period of high metabolism, which water is suddenly eliminated during the last hour of the high metabolism." In the case of the 50 gm. doses the period of high metabolism ended in the same hour that absorption was completed. From the present experiments it is apparent also that the period of high diuresis comes promptly when the flow of glucose into the blood is ended. Therefore, no doubt, as Lusk assumed, the end of the high metabolism and the onset of high diuresis following the administration of 75 gm. of glucose also marked the end of absorption as directly observed in the case of the smaller doses. Then taking the dog's weight at 7.3 kg.[15] and the time as 5 hours, the rate of absorption would have been 1.6 gm. per kg. per hour as against 1.7 gm. reckoned in the case of the 50 gm. doses. The fact that the metabolism was increased to the same level with the 50 and 75 gm. doses in the same animal confirms the reckoning which shows that the same absorption rate prevailed in the two cases.

Interpretation of the Effects of Glucose on the Water Balance.

The retention of water was attributed by Lusk and by Fisher and Wishart to the increased osmotic power of the organism due to the presence of increased amounts of freely diffusible sugar. Following the alimentary administration of 50 gm. of glucose in 150 cc. of water they found during the 1st hour a rapid absorption of glucose into the blood but no fall of the hemoglobin percentage to indicate hydremia. It was presumed that in this hour the balance of glucose still in the alimentary tract held water back. But by the end of the 2nd hour, two-thirds to three-quarters of the glucose having been absorbed, they found evidences of hydremia, and in the 4th hour (absorption completed) hydremia passed and hydruria came. Taken in conjunction with well known observations that direct intravenous injection of glucose may lead promptly to hydremic plethora and that glucose so injected passes with great rapidity from the blood into the tissues where it promptly ceases to exist as such, this presents the picture of a tide of glucose and water molecules moving together through the organism, the glucose finally disappearing within the cells and the water into the urine.

At the present time it might seem preferable to express the matter in terms other than those of the osmotic theory. Other-

[15] The experiment of March 27, 1912, Lusk,[12] pp. 44–45.

wise the above conception appears adequate to explain the variations of the water balance seen in connection with intravenous injections of glucose. In accordance with the solvate theory,[4] glucose molecules would tend to hydrate themselves, the number of water molecules held by a molecule of glucose depending upon the individual hydration capacity of glucose, on the amount of water present, and on the amounts and individual hydration capacities of other molecules in the solution, etc. During intravenous injections at subtolerant rates when the preponderance of all the free glucose in the body is in the blood, its hydration should lead to hydremia and this to decreased diuresis, unless an excess of water is injected with the glucose. But when glucose is injected faster than it can be chemically changed in the cells, and so passes through the renal cells and appears on the urinary side of this membrane, water must tend to accumulate there also; how much, should depend upon the quantitative distribution of glucose between the blood and urine phases, on the total supply of water in the body, etc.

This would imply that the diuretic effects of glucose are an expression of the power of the glucose molecule to hydrate itself, and of the presence of hydrated glucose on the urinary side of the renal cell. Hofmeister[16] attributed the power of salts to dehydrate colloid gels to the tendency of the salt to hydrate itself. Fischer[17] has drawn a parallel between the diuretic effects of salts and their power to dehydrate protein gels, and made experiments tending to show that the parallel holds for sugar. The above interpretation is therefore in harmony with the view previously expressed, although differing in detail.

The present results and interpretation are contrary to the generality of Allen,[18] that all the common sugars are diuretics when given intravenously, that the phenomenon of water retention during glucose administration is observed only when the sugar is given by the alimentary, subcutaneous, or other indirect route, and contrary also to his conception that antidiuresis is dependent upon the union of glucose with a colloidal substance, etc.

[16] Hofmeister, F., *Arch. exp. Path. u. Pharm.*, 1890, xxvii, 395.

[17] Fischer, M. H., Edema and Nephritis, New York, 2nd edition, 1915.

[18] Allen, F. M., Studies on Glycosuria and Diabetes, Cambridge, 1913, 1052–4.

SUMMARY AND CONCLUSIONS.

1. The technique employed in making continued injections of glucose and water into the veins of animals (rabbits, dogs) at known rates by means of a previously described volumetric pump is reported in detail, with a note on the purification of commercial glucose.

2. Experiments are cited to show that the maximum rate at which glucose can be administered continuously by vein to normal resting rabbits and dogs without causing gross glycosuria (*i.e.*, the normal intravenous glucose tolerance limit) is close to 0.85 gm. per kg. per hour in the great majority of cases, but may be found, more frequently than in man, a little higher (0.9 gm. per kg. per hour). Within rather wide limits the water administered simultaneously with the glucose may be varied without appreciably changing the rate of glucose utilization.

3. Continued intravenous injections of glucose at uniform rates between 0.9 and 2.0 gm. per kg. per hour may lead to continued excretions of glucose in the urine at uniform rates. The ratio of the intake to the output rate differs with different individuals receiving glucose at the same rate, and with different rates of injection in the same individual. When the injection rate is 1.8 to 2.0 gm. per kg. per hour, the glucose excretion per hour has represented as little as 2 and in one case as much as 10 per cent of the injection. Within rather wide limits variation of the quantity of water injected and eliminated during constant glucose injections at these rates has not been found to affect the rate of glucose utilization or excretion.

4. For any individual case the rate at which glucose enters the blood determines the rate of glucose utilization and excretion regardless of the volume of the blood and urine or the concentration of glucose in them.

5. The observed constancy of the velocities of glucose metabolism and excretion, in spite of such changes, is explained on the basis that water must so distribute itself in the body that the ratio of the plasma volume to its surface of contact with the cells at large (specific surface of the plasma phase) remains constant. The terms plasma and cells are employed in the general sense of phases in heterogeneous systems.

6. By controlling the rates of glucose and water injection it has been found possible to produce relative and absolute retardations of diuresis during glucose injections at rates causing glycosuria, as well as with those at subtolerant rates. But acceleration of the rates of water elimination (over and above that ascribable to the water given with the glucose) has not been observed without glycosuria.

7. The tendency toward water retention is attributed to the presence of free diffusible glucose in the blood and, to a less extent, in the tissues and the tendency of the glucose to hydrate itself in accordance with the solvate theory. The tendency toward diuresis, manifest only when glucose gains access to the urine, is regarded as an expression of the same process operative in another place. Where the balance of water will be found in the body during injections of glucose will depend at any moment on the quantitative distribution of glucose among the phases of the organism. Increased diuresis occurs when there is glycosuria and a sufficient supply of water in the organism.

8. Attention is called to the probable fallacy of considering the concentration of any substance in the blood plasma as a reliable index *per se* of the rate at which it enters the cells, of the rate of its utilization or excretion, or of the toxic effects produced.

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INTRAVENOUS INJECTIONS OF β-HYDROXYBUTYRIC AND ACETOACETIC ACIDS.

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β-Hydroxybutyric Acid.

The sodium salt of *l*,β-hydroxybutyric acid in 2 to 3 per cent solution was injected intravenously into normal, well nourished, resting dogs at selected rates maintained uniformly during periods of 2 hours. The urine excreted during the injections was examined for acetone, acetoacetic acid, and *l*,β-hydroxybutyric acid.

With injections of sodium *l*,β-hydroxybutyrate at the rate of 0.3 gm. (0.0024 gm. mol.) per kg. of body weight per hour, no optically active hydroxybutyric acid became demonstrable in the urine (one experiment). With the injection of 0.4 gm. (0.0032 gm. mol.) per kg. per hour, a trace of *l*,β-hydroxybutyric acid was excreted (one experiment); that is, in the case of a dog weighing 6.35 kg. receiving 2.54 gm. of the sodium salt per hour for 2 hours the rotation of the residue obtained by ether extraction of the total urine, when made up to 10 cc. and read in a 10 cm. tube, was −0.08°, corresponding to about 0.033 gm. of the acid. In an experiment with another animal 0.4 gm. of the sodium salt per kg. per hour caused no detectable excretion of levorotatory substance. With an injection of 0.5 gm. (0.0040 gm. mol.) per kg. per hour in the case of a 6.13 kg. dog, 0.050 gm. of *l*,β-hydroxybutyric acid was recovered in the urine. The sodium nitroprusside-ammonia tests for acetone and the Gerhardt ferric chloride reaction for acetoacetic acid proved negative in each of these urines.

These experiments indicate a normal intravenous tolerance limit[1] for sodium *l,β*-hydroxybutyrate at or about 0.0032 gm. mol. of the salt, or 0.0026 gm. mol. of the free acid per kg. of body weight per hour. The appearance of *l,β*-hydroxybutyric acid in the urine of a dog might therefore be taken to indicate that its rate of entry into the blood exceeds this value, provided the power of the organism to utilize β-hydroxybutyric acid is not diminished.

Acetoacetic Acid.

Experiments performed in an identical manner with the sodium salt of acetoacetic acid resulted as follows: Injections at the rate of 0.1 gm. (0.0008 gm. mol.) per kg. per hour did not lead to the appearance of acetone, acetoacetic acid, or β-hydroxybutyric acid in the urine (two experiments). With injections of 0.2 gm. (0.0016 gm. mol.) per kg. per hour, acetone and acetoacetic acid became demonstrable in the urine, but there was no excretion of β-hydroxybutyric acid (three experiments). An injection of 0.3 gm. (0.0024 gm. mol.) per kg. per hour, in one case, and of 0.4 gm. (0.0032 gm. mol.) per kg. per hour in another, also led to the excretion of traces of acetone and acetoacetic acid, but no *l,β*-hydroxybutyric acid. However, in the case of another dog receiving 0.4 gm. (0.0032 gm. mol.) per kg. per hour *l,β*-hydroxybutyric acid was detected in the urine, and following an injection of 0.5 gm. per kg. per hour in a dog weighing 5.1 kg., 0.041 gm. of *l,β*-hydroxybutyric acid appeared in the urine. With higher rates of injection of sodium acetoacetate proportionately greater amounts of *l,β*-hydroxybutyric acid were recovered.

[1] The rate at which a substance such as glucose or β-hydroxybutyric acid must be introduced into the blood from without in order to cause the substance to appear in the urine does not of necessity represent the rate at which the substance must enter the blood from all sources, endogenous as well as exogenous, to produce this effect. For during the intravenous injection of a substance at a rate a, the same substance may theoretically be entering the blood from endogenous sources at a rate x, so that the actual rate of entry which just suffices to cause the substance to appear unchanged in the urine would be $a + x$. The term *intravenous tolerance limit* is used simply to designate the rate of intravenous injection which just suffices to cause an overflow into the urine of the substance injected.

DISCUSSION.

In these experiments *l*,β-hydroxybutyric acid became demonstrable in the urine when acetoacetic acid was brought into the systemic venous blood at a rate of 0.0032 gm. mol. of the sodium salt per kg. per hour, or at the same rate at which the sodium salt of *l*,β-hydroxybutyric acid itself had to be injected by the same route in order to cause a demonstrable excretion of this acid. It would therefore appear that, in so far as the excretion of hydroxybutyric acid is concerned, the intravenous injection of sodium acetoacetate is equivalent to the injection of the same number of molecules of the former acid itself, which strongly suggests that acetoacetic acid when injected into the systemic blood stream under the conditions of the experiments described, *is reduced almost quantitatively into the hydroxy acid and at a velocity not less than that of the injection*, because were this not the case, the rate of formation of β-hydroxybutyric acid would not suffice to overstep the tolerance limit for this substance as above established.

Two objections might be raised against this interpretation: (*a*) During the injections of acetoacetic acid at the rates necessary to produce detectable excretions of *l*,β-hydroxybutyric acid in the urine, some acetoacetic acid was excreted as such and in addition some came out as acetone. However, the quantities so accounted for were too small to affect the argument. Thus a dog which weighed 12.5 kg. received in 2 hours an actual total of 10 gm. of sodium acetoacetate, and from one-half the total urine collected during the injection period there was recovered 0.027 gm. of acetone, corresponding to a total excretion for the period of 0.095 gm. of acetoacetic acid or about 1 per cent of the material injected. (*b*) There is the possibility that the observed excretions of β-hydroxybutyric acid coincident with injections of sodium acetoacetate might arise from indirect effects of the acetoacetate, such as a diminution of the rate of utilization of endogenous β-hydroxybutyric acid, an increased endogenous supply of the latter substance, or some unforeseen disturbance of metabolism. This possibility has not been fully excluded. A control was made with sodium butyrate administered at the rate of 0.75 gm. per kg. per hour for 1 hour and in this case no β-hydroxybutyric acid, acetoacetic acid, or acetone was found in the

urine. Moreover the administration of sodium acetoacetate at rates below 0.4 gm. per kg. per hour failed to cause any excretion of β-hydroxybutyric acid.

The experiments further directly suggest that during the injection of *l*,β-hydroxybutyric acid at rates up to 0.5 gm. per kg. per hour, less than 40 per cent at most of the hydroxy acid can be converted into acetoacetic acid because the tolerance limit for the latter substance, in the form of its sodium salt, lies below 0.2 gm. (0.0016 gm. mol.) per kg. per hour and injections of sodium β-hydroxybutyric acid at 0.5 gm. (0.0040 gm. mol.) per kg. per hour were not accompanied by any excretion of acetoacetic acid. There is indeed no direct evidence in these experiments that any of the injected hydroxybutyric acid was oxidized to acetoacetic acid and the evidence obtained by injections of acetoacetic acid indicates that if any of this acid were formed it would necessarily be reconverted into the hydroxy acid. It would appear therefore that if acetoacetic acid and hydroxybutyric acid are in equilibrium in the organism, as held by Neubauer and others, the balance in health is swung heavily toward the hydroxybutyric side.

In 1910 Blum[2] reported the excretion of *l*,β-hydroxybutyric acid in the urine following sufficiently large subcutaneous injections of the acetoacetic acid sodium salt, and almost simultaneously Dakin[3] reported similar results following intravenous injections as well. Marriott[4] was able to demonstrate an increase of the β-hydroxybutyric acid concentration in the blood and tissues and its excretion in the urine following intravenous administrations of sodium acetoacetate but was not successful in demonstrating the conversion of β-hydroxybutyric acid into acetoacetic acid. The results of the present experiments confirm these observations and add data of a quantitative character. Incidentally they serve to illustrate the use of the method of timed intravenous injections in a problem of intermediate metabolism.

In a later paper we hope to report on the relationships between acetoacetic and β-hydroxybutyric acids under other metabolic conditions.

[2] Blum, L., *Münch. med. Woch.*, 1910, lvii, 683.
[3] Dakin, H. D., *J. Biol. Chem.*, 1910, viii, 97.
[4] Marriott, W. M., *J. Biol. Chem.*, 1914, xviii, 241.

CONCLUSIONS.

I. *l,β*-Hydroxybutyric acid became demonstrable in the urine of normal dogs receiving continuous intravenous injections of sodium *l,β*-hydroxybutyrate at the rate of 0.4 gm. (0.0032 gm. mol.) per kg. of body weight per hour for 2 hours, but not with lower rates. Sodium acetoacetate appeared in the urine when injected for the same time at the rate of 0.2 gm. (0.0016 gm. mol.) per kg. per hour, but not with lower rates.

II. Sodium acetoacetate when injected at rates close to 0.4 gm. (0.0032 gm. mol.) per kg. per hour gave rise to the excretion in the urine of *l,β*-hydroxybutyric acid.

III. Since the number of molecules of sodium acetoacetate required per kg. of body weight per hour to cause the appearance of *l,β*-hydroxybutyric acid in the urine did not differ appreciably from the number of molecules of the sodium salt of *l,β*-hydroxybutyric acid itself which were necessary to produce the same effect, it would appear most probable that acetoacetic acid, under the conditions of these experiments, is converted almost quantitatively into *l,β*-hydroxybutyric acid.

IV. The experiments afford no direct evidence to indicate that any of the injected *l,β*-hydroxybutyric acid was oxidized to acetoacetic acid.

EXPERIMENTAL.

Material.—*l,β*-Hydroxybutyric acid was obtained by ether extraction of diabetic urine which had been concentrated by evaporation. The calcium zinc salt of the acid was made from this extract by Shaffer's method[5] and purified by recrystallization. From the pure salt the free acid was obtained by acidifying with phosphoric acid and ether extraction. The residue from the ether was dissolved in water and the acid determined by titration and polariscope. The entire amount was neutralized with sodium carbonate. Sodium chloride and water were then added to give solutions for injection containing from 2 to 3 per cent sodium *l,β*-hydroxybutyric acid and 0.5 per cent sodium chloride.

[5] Shaffer, P. A., and Marriott, W. M., *J. Biol. Chem.*, 1913–14, xvi, 265.

Acetoacetic acid was prepared from its ethyl ester after the method of Ceresole.[6] The ester was saponified, the solution then saturated with ammonium sulfate, and extracted with ether. The ether residue was neutralized with barium carbonate and the unchanged ester removed by extraction with ether. The acetoacetic acid was then freed with sulfuric acid and extracted with ether. The ether residue was dissolved in water and the acid content of this solution determined by titration and as acetone. Sodium carbonate, in amount just sufficient to neutralize, and sodium chloride and water were added so that the solutions for injection contained from 2 to 5 per cent of sodium acetoacetate and 0.5 per cent of sodium chloride. The acetoacetic acid was freshly prepared for each experiment.

Animals.—Uniform injection rates were secured by means of a volumetric pump described by Woodyatt.[7] The volumes actually injected in the various experiments ranged from 31 to 124 cc. per hour and never deviated more than 1 or 2 per cent from the volume which it was desired to give. There was no difficulty in maintaining uniform rates. In each experiment the following procedure was adhered to: The dog was made as comfortable as possible on a padded animal board and a superficial vein of the leg was exposed under 0.5 per cent cocaine. An hour later the bladder was emptied by catheter and a 2 hour fore-period commenced. At the end of this period a cannula was gently slipped into the vein, the bladder emptied and irrigated, and the injection started. After 2 hours the bladder was again emptied and the injection stopped. In some cases urine was collected for an after-period of 2 hours, the dog remaining on the table.

Analyses.—The urines were examined for acetone by the sodium nitroprusside-ammonia test and for acetoacetic acid by the Gerhardt ferric chloride reaction. Free acetone was determined by aeration into standard iodine by Folin's method, and acetoacetic acid was titrated as acetone, after distillation, by a conventional iodine thiosulfate method. *l*,β-Hydroxybutyric acid was determined by ether extraction and polariscope according to Bergell.

[6] Ceresole, *Ber. chem. Ges.*, 1882, xv, 1327.

[7] Woodyatt, R. T., *J. Biol. Chem.*, 1917, xxix, 355.

TABLE I.

Intravenous Injections of Sodium l,β-Hydroxybutyrate.

Experiment.	Date.	Dog	Body weight.	Injection.			Urine findings.		
				Rate per kg per hour.	Duration.	Total injected.	Nitroprusside test.	Gerhardt test.	*l,β*-Hydroxybutyric acid.
	1917		*kg*	*gm.*	*hrs.*	*gm.*			*gm.*
1	March 9	A.	6.60	0.30	2	3.96	0	0	0
2	" 15	"	6.35	0.40	2	5.09	0	0	0.033
3	" 27	B.	5.12	0.40	2	4.10	0	0	0
4	" 21	A.	6.13	0.50	2	6.13	0	0	0.050

TABLE II.

Intravenous Injections of Sodium Acetoacetate.

Experiment.	Date.	Dog.	Body weight.	Injection.			Urine findings.				
				Rate per kg. per hour.	Duration.	Total injected.	Nitroprusside test.	Gerhardt test.	Free acetone.	Acetone and acetoacetic acid.*	*l,β*-Hydroxybutyric acid
			kg.	*gm.*	*hrs.*	*gm.*			*gm.*	*gm.*	*gm.*
5	May 11, 1916	C.	9.70	0.10	2.0	1.94	0	0	—	—	—
				0.20	0.5	3.88	+	+	—	—	—
6	April 18, 1917	D.	11.10	0.10	2.0	2.22	0	0	—	0.005	—
				0.20	2.0	4.44	+	+	—	0.015	—
7	" 18, 1917	E.	13.40	0.30	2.0	8.04	+	+	0.006	0.033	0
8	" 8, 1917	F.	12.50	0.40	2.0	10.00	+	+	—	0.095	0
9	June 1, 1916	G.	9.66	0.40	2.0	7.72	+	+	—	—	0.028
10	April 8, 1917	B.	5.10	0.50	2.0	5.10	+	+	—	—	0.041
11	May 25, 1916	C.	9.55	0.70	3.0	13.75	++	++	—	0.547	0.414
12	June 21, 1916	H.	8.63	0.85	2.0	14.68	++	++	—	—	0.514

* Calculated in terms of acetoacetic acid.

RAPID REDUCTION OF INTRA-OCULAR TENSION IN GLAUCOMA

BY TIMED INTRAVENOUS GLUCOSE INJECTIONS *

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In the course of studies with timed intravenous injections of sugars, salts, etc., several phases of which have been previously reported by Woodyatt, Sansum and Wilder,[1] observations have been made on possible clinical uses of the rapid dehydration of the organism which may be produced when sugar or salts are injected uniformly into the vein at rates which lead to the excretion of water through the kidneys faster than water is administered with the sugar or salt.

When glucose is injected by vein uniformly at or below the tolerance limit which, in normal resting individuals, lies as a rule between 0.8 and 0.9 gm. per kilogram of body weight per hour, there is no increase in the rate of diuresis greater than can be accounted for by the extra water given.[2] In fact, a definite antidiuretic tendency manifests itself.[3] But when glucose is injected so fast as to produce a sufficiently rapid excretion of glucose, increased diuresis results; and then if the water injected with the glucose is less than that excreted in the urine, the tendency is toward dehydration. By employing 36 to 54 per cent. glucose solutions and injecting them at rates corresponding to from 3.6 to 5.4 gm. of glucose per kilogram per hour, heavy glycosuria and rapid dehydration occur.

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1. Woodyatt, R. T., Sansum, W. D., and Wilder, R. M.: Prolonged and Accurately Timed Intravenous Injections of Sugar, THE JOURNAL A. M. A., Dec. 11, 1915, p. 2067. Woodyatt, R. T.: Studies in Intermediate Carbohydrate Metabolism, Harvey Society Lecture, 1916.

2. Wilder, R. M., and Sansum, W. D.: d-Glucose Tolerance in Health and Disease, Arch. Int. Med., February, 1917, p. 311.

3. Woodyatt, R. T., and Sansum, W. D.: Jour. Biol. Chem., 1917, **30**, 155.

The question arises as to whether or not and to what degree fluid can be drawn to advantage from a localized area such, for example, as a blister, an effusion in a serous cavity, a glaucomatous eye, or the like. An opportunity for experimental study presented itself when Dr. E. V. L. Brown asked that we attempt to lower the intra-ocular tension in a certain case of glaucoma then under his observation. Three successful attempts with this case led to the application of the same principle in a second case on the service of Dr. William H. Wilder. The results obtained are reported in the belief that the principles and methods employed will be found of clinical interest. It is not improbable, however, that the details of the operation as here described might to advantage be modified, since it would appear that the dehydration was unnecessarily rapid and that equivalent beneficial results might be attained with less risk of harm by a more gradual procedure.

REVIEW OF THE LITERATURE

The abstraction of fluid from the body as a whole, including the eye, is by no means a new idea in the management of glaucoma, for among the accepted treatments are free elimination by sweating, diuresis and catharsis. Local abstractions of fluid by the application of leeches or the subconjunctival injection of salts are also well known procedures. According to Fischer's[4] theory of glaucoma, the condition constitutes essentially a local edema, a swelling of the ocular colloids, especially of the vitreous, due to an increased affinity of the colloids for water. As salts in suitable concentrations may cause the separation of water from swollen protein gels, so in an analogous manner they may lower the tension of an eye. On the basis of experiments showing the parallel effects of salts on enucleated eyes and on protein gels, Hayward and Fischer[5] suggested both local and general treatments, the former consisting in subconjunctival injections of from eighth molecular to sixth molecular (from 4.05 to 5.41 per cent.) sodium acetate solutions, the latter in rectal administration of from 1,000 to 1,500 c.c. of

4. Fischer, M. H.: Arch. f. d. ges. Physiol. (Pflüger's), 1908, **125**, 396; 1909, **127**, 1.
5. Hayward, G. T., and Fischer, M. H.: Ann. Ophth., 1910, **19**, 40.

Fischer's hypertonic salt solution. Both in their hands gave beneficial results. Hertel[6] used hypertonic salt solutions to lower intra-ocular tension as a preliminary step to iridectomy. He reports one case in which, following the intravenous injection of 120 c.c. of a 10 per cent. solution, the intra-ocular tension fell from 72 to 30 mm. of mercury. The time or rate of injection was not stated. In another case following the oral administration of 200 c.c. of the same solution, the tension was lowered from 80 to 22 mm. of mercury. He also used hypotonic solutions to restore ocular tension in the hypotonia found in such conditions as diabetic coma. In this connection it is interesting to note in anticipation of what follows that the "soft eyeball" which may be encountered in diabetes would appear to be merely a part of that general dehydration of the body which goes hand in hand with sugar diuresis and other manifestations of the effects of excessive quantities of free glucose in the body.

TECHNIC

The glucose employed was a chemically pure product prepared in this laboratory. The details of its preparation are described elsewhere.[3] The water was freshly distilled with the same precautions as are observed in its preparation for salvarsan administrations. Approximately 2 to 3 molecular (36 to 54 per cent.) glucose solutions were made up by weight, carefully filtered and sterilized in the autoclave. The exact titer was then determined in each case by the polariscope. The volumetric pump has been described in detail in a recent communication.[7] The details of operation, with the arrangement of the apparatus, also have been described elsewhere.[2] The rate of injection will depend on the titer of the solution used, the nature of the case, and the quantity of water it is desired to abstract from the body in a specified time. The slower rates of injection extending over two hours or more tend to eliminate the chill and lessen the dangers attendant on the too rapid increase of intravascular pressure and decrease of intra-ocular tension. Other things being equal, the higher the rate of glucose injection and the less the water given with

6. Hertel: Arch. f. Ophth. (Sattler), 1915, **90**, 309.
7. Woodyatt, R. T.: Jour. Biol. Chem., 1917, **29**, 355.

the glucose, the greater the dehydrating power. A 54 per cent. solution given at a rate corresponding to 3.6 gm. of glucose per kilogram per hour (that is, 6.66 c.c. of a 54 per cent. solution per kilogram per hour) has greater dehydrating power than the same quantity of glucose given in the same time in the form of a 36 per cent. solution (that is, 10 c.c. of a 36 per cent. solution per kilogram per hour). With a solution of fixed concentration (as 54 per cent.), the degree of dehydration will be proportional to the quantity given in a specified time. The intra-ocular tension was taken with a Gradle-Schiötz tonometer by the ophthalmologists in charge of the case.

CASE HISTORIES AND EXPERIMENTS

CASE 1.—This was one of syphilitic iritis plastica with secondary glaucoma on the service of Dr. E. V. L. Brown, who provided the following history:

History.—Mrs. S., colored, widow, aged 52, was admitted to the Cook County Hospital, Dec. 1, 1915, complaining of pain and swelling in the left eye, photophobia, headaches, joint pains and a fading maculopapular eruption on the body. The eye trouble had begun about two weeks before admission with redness and photophobia in the left eye followed by failing vision and joint pains which grew progressively worse. The rash, accompanied by sores in the mouth and headaches, had appeared about six weeks prior to admission. The past history was negative.

Physical Examination.—The patient is a fairly well developed but poorly nourished negress. She appears ill and as if she had recently lost in weight and strength.

Eyes: There is slight edema of the conjunctiva of the right eye. The left eye is nearly closed, owing to edema of the lids. There is profuse lacrimation and chemosis of the bulbar conjunctiva. The cornea is dulled and everywhere overstrewn with epithelial pits. The stroma is clear. There is no gross keratitis punctata. There is marked ciliary and conjunctival injection. Two thirds of the anterior chamber is obliterated totally in the sector corresponding to that between I and VII of the clock dial, and half obliterated in the sector between VII and I by the anteriorly displaced iris. The central one fourth of the chamber is normal in depth. The very irregular, 2 mm. pupil is completely encircled by posterior synechias, and is blocked by a greenish white, nonvascular exudate. There is a sail-like forward bulging of the body of the iris (*iris bombe*). There are no condylomas or tuberculous nodes, although the iris is furrowed radially from the pupillary border one-half way to II of the clock

dial and less deeply to XI. The iris markings are blurred, but otherwise the tissue is not much changed. The crystalline lens and entire back of the cornea are covered by very fine discrete gray flecks of exudate. R. V., 3/10; L. V., shadows. Tension, right, 18¾; left, 54 mm. of mercury. The synechiae are uninfluenced by the use of atropin.

Mouth: One small area suggests a mucous patch. Neck: There is cervical and submaxillary adenopathy. Chest: Negative. The heart tones and borders are normal. Blood pressure, 135 systolic and 80 diastolic. Genitalia: There is inguinal and femoral adenopathy. Extremities: There is marked epitrochlear adenopathy. Skin: Across the forehead, about the eyes, and on the upper forearms are numerous split-pea sized, flat, slightly elevated papules which appear a little darker than the normal skin. Laboratory: Urine, normal. Wassermann reaction in the blood plasma + + +. Complement fixation test for gonorrheal infection in the blood plasma +.

Therapeutic Dehydration.—An iridectomy for the immediate relief of pressure would have been the procedure of choice; but, owing to the fear of complications, such as prolapse of the iris and intra-ocular hemorrhage from the too sudden lowering of tension, the surgeon preferred, if possible, to operate under more favorable conditions. The proposal was therefore made to attempt the reduction of intra-ocular tension by general means. December 6, after a careful physical examination had demonstrated a sound cardiovascular-renal system with the blood pressure within normal limits, the patient received 41.2 per cent. glucose solution by vein at the rate of 11.2 c.c. per kilogram per hour for sixty-nine minutes. The 47.5 kg. patient thus received in sixty-nine minutes a total of 612 c.c. of 41.2 per cent. glucose solution containing 252 gm. of glucose and approximately 471 c.c. of water. As the result of the injection, the patient passed 2,247 c.c. of urine, as against the 471 c.c. of water injected with the glucose, thereby creating a water deficit in the body of 1,776 c.c., or 37.4 c.c. per kilogram. The blood pressure before the operation was 135 systolic and 80 diastolic. During the injection it rose to 155 — 100, and after the injection it fell to 95 — 65, systolic and diastolic, respectively. On the following morning at 10: 30 it had risen to 120 systolic and 90 diastolic. The temperature before the injection was 98.4 F. One hour after the injection it was 102. It then gradually declined to normal in the course of twenty-four hours. Toward the end of the injection for twenty minutes the patient shook as though in a chill, but did not complain of any discomfort. The intra-ocular tension before the injection was 60.5 mm. of mercury and fell to 26 mm. In the right or normal eye it fell from 15.25 to 12.25. The fall in tension was so striking that the question was raised as to whether if the operation were repeated and

followed by a suitable after-treatment the advantage gained might not be maintained for a long enough time to preclude the necessity of operation entirely.

Second Dehydration.—December 14, the treatment was repeated. This time an effort was made to abstract the water more gradually. The patient received a 38.9 per cent. glucose solution by vein at the rate of 8.7 c.c. per kilogram per hour for 108 minutes. The 45 kg. patient thus received in 108 minutes 702 c.c. of 38.9 per cent. solution containing 273 gm. of glucose and approximately 548 c.c. of water. As a result of the injection the patient passed 2,359 c.c. of urine while receiving 548 c.c. of water with the glucose, thereby creating a water deficit in the body of 1,811 c.c., or 40 c.c. per kilogram. The tension in the left eye fell from 54 to 18 mm. of mercury. In the right or normal eye it fell from 15.25 to 7.25. In this instance the blood pressure reached a maximum of 185 mm. There was a "chill" lasting forty minutes. The temperature rose to 102.2. The after-treatment consisted in the limitation of the total water intake to 2,000 c.c. in twenty-four hours, with fifteen doses each consisting of 1 gm. of sodium bicarbonate and 0.66 gm. of sodium acetate with compound jalap powder and magnesium sulphate enough to produce three or four watery stools daily. The after-treatment failed. The patient complained of thirst, and the intra-ocular tension gradually rose, reaching 44 mm. of mercury by the end of twenty-four hours, when the treatment was stopped because of nausea. After this the tension gradually rose, reaching 47 in three days and 66.75 by the end of a week.

Third Dehydration.—This was performed, December 21, as a preliminary measure to an iridectomy. The procedure was carried out still more slowly than in the second injection. The patient received 36 per cent. glucose solution at the rate of 7.7 c.c. per kilogram per hour for 115 minutes. Thus the 45 kg. patient was given 662 c.c. of 36 per cent. solution, containing 238 gm. of glucose and approximately 530 c.c. of water. The urine totaled 2,275 c.c., while 530 c.c. of water were injected with the glucose. A water deficit of about 1,745 c.c., or 39 c.c. per kilogram, was therefore produced. Although nearly as much water as before was abstracted, the time consumed was longer, and there was in this instance no chill. The temperature rose to 101. The systolic blood pressure reached 148 and the diastolic 100 mm. of mercury. The intra-ocular tension was reduced from 66.75 to 37.75. This reading was taken just before the close of the injection; but at the time of operation, thirty minutes later, it was normal to palpation. Iridectomy was then performed by Dr. Brown. The patient was put on an antisyphilitic management and made an uneventful recovery. The cornea became clear. The hyperemia and the anterior synechiae disappeared. The tension remained so low that it was impossible to make satisfactory readings.

The foregoing results were taken to demonstrate the feasibility of lowering intra-ocular tension in a case of the type described; but the question remained as to whether or not parallel results could be obtained in a case of so-called primary glaucoma.

Case 2.—This was one of very severe acute, primary glaucoma that developed after the patient had been in the hospital about a month and in spite of careful medicinal and surgical management. The patient was one of Dr. W. H. Wilder, who kindly furnished the history.

History.—Mrs. Jane G., aged 46, with glaucoma, was first examined by me, Jan. 1, 1916. She gave a history that one and a half years before the sight of the right eye began to fail, and examination by a specialist revealed that the fields were contracted. About June 1, 1916, she noticed that the central vision of the right eye was much reduced. At times she has had pain in the eyes and has noticed rainbows about a light. Recently the sight of the left eye has been clouded, especially so when she feels nervous.

R. V. with sph. 6.00, cyl. 2.00, axis 90° 20/70
L. V. with sph. 6.50, cyl. 1.75, axis 110° 20/30

Disks: The right is deeply cupped, about 2 mm. In the left there is a deep physiologic cup, but the edges are normal. The vessels are somewhat congested. The intra-ocular tension in the right eye is 55 and in the left 25 mm. of mercury. Fields: It is impossible to chart the fields of the right eye. In the left eye the white is somewhat contracted above and below. The red reads: above, 18°; below, 18°; nasal, 18°, and temporal, 55°. The green reads: above, 10°; below, 10°; nasal, 12°, and temporal, 40°.

I advised an operation on the right eye and later on the left eye if the tension should become increased. June 26, 1916, at the Presbyterian Hospital I did an Elliot trephining on the right eye with good results. About two weeks later the tension in the left eye became high and the patient had an acute attack of glaucoma in this eye. July 14, I did an iridotasis on the left eye and at the same time an iridectomy on the right eye, for the tension in that had risen again. The right eye quieted down following this iridectomy. The left eye recovered well from the iridotasis and remained low or normal for a few days, when the patient again had an acute attack of glaucoma with a tension up to 60 mm. of mercury, shallow anterior chamber, and violent pain requiring narcotics.

Therapeutic Dehydration.—An iridectomy for the immediate relief of pressure would have been the operation of choice; owing, however, to the fear of complications, such as prolapse of the iris and intra-ocular hemorrhage from the too sudden lowering of tension, the surgeon preferred to work

under more favorable conditions. Knowing of the work on the previous case, Dr. Wilder asked that we attempt to lower the tension in this case by the intravenous injection of glucose in preparation for an iridectomy. A careful physical examination showed that the heart, blood vessels and urinary system were in sufficiently good condition. The systolic blood pressure was 146 and the diastolic 100 mm. of mercury. There was a marked edema of the lids and surrounding tissues. The intra-ocular tension was 60 mm. The anterior chamber was obliterated. July 20, the patient received 55.2 per cent. glucose solution by vein at the uniform rate of 10 c.c. per kilogram per hour for seventy-six minutes. The 60 kg. patient thus received 691 c.c. of 55.2 per cent. glucose solution containing 380 gm. of glucose and approximately 491 c.c. of water. As a result of the injection there was passed 1,250 c.c. of urine as against 491 c.c. of water injected with the glucose, thereby creating a water deficit in the body of 759 c.c., or 13 c.c. per kilogram. The intra-ocular tension as observed by Dr. W. H. Wilder fell from 60 to 20 mm. of mercury. There was no chill. The temperature rose to a maximum of 101. The iridectomy was then performed by Dr. Wilder under light ether anesthesia. Before and during the first part of the injection the patient suffered greatly from pain in the eye and was very restless. During the latter part the pain wholly disappeared, and the patient became quiet and went to sleep. *The edema of the lids and surrounding tissues disappeared entirely under the eyes of the observers during the injection.* The anterior chamber remained very shallow. The high rate of injection was chosen in this instance because the patient had been taking large doses of morphin which it was feared might retard the liberation of water, although it was not known by experiment that such would be the case. Dr. Wilder provides the following notes on the subsequent clinical history of the case:

Subsequent History.—Before the iridectomy, July 20, 1916, the tension was lowered by the intravenous injection of glucose solution by Dr. Sansum and Dr. R. M. Wilder. This lowered the tension to 20 mm. of mercury, and an iridectomy was satisfactorily done under ether narcosis administered by Dr. Herb. The eye quieted after this operation, and in one week the patient left the hospital. The tension remained normal, but the patient continued using 1 per cent. physostigmin (eserin) and 5 per cent. ethylmorphin hydrochlorid (dionin). She was weak for some time, and the elixir of iron, quinin and strychnin was prescribed. The tension seemed increased at times. Feb. 10 1917, it was 35 mm. Feb. 21, 1917, R. V. 0; L. V. with sph. 200, cyl. 5.50, axis 15° $^{20}/_{50}$. These lenses were ordered in tinted glass and the physostigmin and ethylmorphin hydrochlorid continued. The right optical disk was deeply cupped and the left slightly cupped.

COMMENT

The theory of the nature and mechanism of glucose diuresis has been discussed elsewhere[3] and need not be repeated here. The increased temperature observed with each of the injections is a phenomenon which we have encountered in the course of intravenous glucose injections in animals when dehydration has been pushed beyond a certain degree. The same applies to the chill. A discussion of the theoretical significance of the fever and chill would be irrelevant in this place. The increased blood pressure is associated with the increased blood volume due to the flow of water into the blood owing to the presence in the blood of increased quantities of free glucose.[8]

Attention is directed to the temporary but marked elevation of blood volume and blood pressure during the intravenous injection of glucose because an injudicious application of the principle of intravenous sugar or salt injection to cases in which the cardiovascular system was impaired might lead to deleterious effects. In the cases here reported the treatment was not instituted without a careful preliminary consideration of the heart, vessels and renal function. A powerful procedure such as this, which is capable of radically lowering intra-ocular tension and simultaneously elevating the intravascular tension, should obviously not be undertaken blindly or without due care in cases in which the condition of the vessels, especially those within the eye, would render them liable to rupture by a too rapid change of pressure relations. As stated at the outset, the details of the operation as here described are not presented as necessarily the most appropriate for clinical work generally. They might be modified in other cases and in the light of further experience.

By a suitable selection of the rate of glucose injection and of the quantity of water abstracted in a given time, it should be possible to control to a nicety the rate and degree of ocular pressure reduction to meet the demands of the ophthalmologist. In this connection it may be noted that the possibility of positive control is greater than it is in the ordinary local opera-

8. Compare Lusk: Jour. Biol. Chem., 1912, **13**, 27. Fisher and Wishart: Ibid., 1912, **13**, 49. Woodyatt and Sansum (Footnote 3).

tive procedures which are in general use by ophthalmologists.

The experiments demonstrate without any question the possibility of quickly lowering intra-ocular tension in the types of glaucoma described and in the normal eye.

It is not the purpose of the present report to decide when a procedure of the kind herein described should have the preference over other known resources in ophthalmologic practice. It appears to offer a means of lowering intra-ocular tension to a degree and at a rate chosen by the surgeon either in a normal or a glaucomatous eye. It would appear to be a method particularly sensitive to control and especially fitted to meet the demands for a rapid temporary reduction of intra-ocular tension to prevent the damaging effects of mechanical pressure and to pave the way for a surgical operation which cannot be safely or satisfactorily performed with the existing intra-ocular tension. The advantages or disadvantages of the method as compared with cyclodialysis, sclerotomy, etc., could be stated only after further investigation by specialists.

INTRAVENOUS GLUCOSE INJECTIONS IN SHOCK*

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In accordance with a prevailing opinion, the low blood pressure in "shock" is associated with a diminished effective volume of blood in the vessels, and the two factors constitute in themselves essential parts of the shock mechanism. On this assumption, benefits should result from procedures having the effect of holding water within the vessels. In normal individuals it is possible to accomplish this by effecting an entrance of glucose into the blood at appropriate rates sustained for sufficient lengths of time. Marked hydremic plethora and striking increases of the systolic blood pressure may thus be produced, and the present study has shown that the mean arterial pressure may also be elevated in this way. Glucose is, moreover, a food; it is capable of increasing the power of involuntary muscle contractions, elevating the total metabolism, combating at least one type of acidosis, and restoring depleted glycogen reserves. Has it a value in the treatment of cases of shock?

During the past two years, Woodyatt, with Sansum and Wilder, has studied the physiologic effects of sustained intravenous glucose injections at known rates, and applied the method to several clinical conditions, including two cases having features of shock. Favorable results in these cases made it appear desirable to study a longer series under conditions permitting

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more exact observations. This was not possible with the available clinical material, and it was decided to test the principles in cases of experimental shock in animals. Erlanger and his associates have during the summer performed some sixty experiments on shock in animals with special reference to the circulatory phenomena and the vasomotor mechanism. A large number of records for control purposes and a perfected technic were thus available. The primary purpose of the work here reported was to ascertain how the symptoms of experimental shock in dogs might be influenced by intravenous glucose therapy. Incidental observations were made on the effects of salt solutions, salt and carbonate solutions, and epinephrin. The work was done in eight days, and no attempt is made to give this report the character of a completed study. The possible differences between the conditions induced in dogs and that of shock in human cases is appreciated, as also the fact that the slow intravenous injection of glucose in postoperative conditions is not a new procedure.[1]

PHYSIOLOGIC PRINCIPLES

When a single dose of glucose is injected into a peripheral vein, there is a temporary increase of the total quantity of sugar in circulation. The injected sugar passes rapidly out of the blood into the tissues. During its stay in the vessels, the blood volume rises and a state of hydremic plethora develops, owing doubtless to the binding of water by the extra glucose in the vessels. This is shown by a fall in the hemoglobin percentage, and a coincident fall in the blood sugar percentage following an initial rise. These facts were demonstrated by Brasol[2] in 1884, and confirmed and amplified by Biedl and Kraus,[3] Starling,[4] Kleiner and Meltzer, and others. Similarly during the period of absorption following the alimentary administration of glucose there is at first a rise but later a return to the normal of the blood sugar percentage, and simultaneously with the latter a fall in the hemoglobin percentage.[5] In shock cases it is frequently impracticable

1. Kausch: Deutsch. med. Wchnschr., 1911, **37,** 8.
2. Brasol: Arch. f. Physiol., 1884, p. 211.
3. Biedl and Kraus: Wien. klin. Wchnschr., 1896, **9,** 55.
4. Starling: Jour. Physiol., 1899, **24,** 317.
5. Compare Gilbert and Baudouin: Comp. rend. Soc. de biol., 1908, **65,** 710. Fisher and Wishart: Jour. Biol. Chem., 1912, **13,** 49.

to secure the entrance of glucose into the blood through the alimentary tract, nor is the subcutaneous injection of glucose a harmless clinical procedure. If glucose is injected into the peripheral venous blood of a normal resting (unanesthetized) individual at or below the rate of 0.8 to 0.9 gm. per kilogram of body weight per hour, the injection may be sustained in most cases for hours without causing glycosuria.[6] With injections of 0.9 gm. or more of glucose per kilogram per hour, glycosuria develops, and the rates of sugar excretion then bear definite relationships to the rates of injection. The higher the absolute injection rates, the higher the absolute rates of glycosuria.

During sustained intravenous injections at or below the tolerance rate, glucose passes from the blood into the tissues and there undergoes chemical change (polymerizations, oxidations, etc.) at the same rate as that of injection. There is no material accumulation of glucose itself in the tissues. Injections at subtolerant rates therefore have the effect of increasing the quantity of glucose in the blood without producing any considerable increase of the glucose in the tissues. The result is a disproportionate increase of the power of the blood to hold water, and a state of hydremic plethora develops and presumably persists as long as the injection is sustained. The water held by the blood must come from the tissues or from the injection site or both. If the water injected with the glucose is not sufficient to satisfy the glucose in the blood, some water will flow into the blood from the tissues. When the glucose injection is stopped and the last of the injected glucose passes into the tissues, the water previously held in the blood is to a large extent discharged suddenly into the urine. This whole phenomenon differs essentially from that obtainable with inorganic salt solutions, because such salts when injected also pass into the tissues, but when once there are not destroyed. There is therefore the possibility of salt accumulations in the tissues holding water there, and in part counteracting the effects of the salt in the blood stream.

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6. Woodyatt, R. T.; Sansum, W. D., and Wilder, R. M.: Prolonged and Accurately Timed Intravenous Injections of Sugar, The Journal A. M. A., Dec. 11, 1915, p. 2967. Wilder, R. M., and Sansum, W. D.: d-Glucose Tolerance in Health and Disease, Arch. Int. Med., February, 1917, p. 311. Sansum and Woodyatt: Jour. Biol. Chem., 1917, **30**, 155.

With sustained intravenous glucose injections at rates higher than 0.8 to 0.9 gm. per kilogram per hour, the glucose utilization fails to keep pace with that of injection and the tendency is toward some accumulation of unchanged glucose. If the kidneys are functioning actively this glucose appears on the urinary side of the renal membrane and tends to collect water in this locality, probably for the same reason that glucose in the blood produces hydremia.[7] This tendency is expressed as polyuria. Now if the diuresis exceeds the rate of water administration, water will flow from the tissues to the blood and thence to the urine, and hydremia will be sustained, as long as the water supply is adequate. But if the kidneys do not function in this way, most of the unchanged fraction of the glucose injected remains in the body and tends to behave like a salt, causing accumulations of water in the tissues and body spaces wherever it may go. In shock cases the secretion of urine is likely to be impaired. Accordingly the most rational application of intravenous glucose injections in shock would appear *a priori* and on theoretical grounds to consist in long sustained uniform injections at subtolerant rates. Just what the tolerance limit will be in any pathologic condition cannot be stated. When the kidney function is impaired the rate of injection necessary to produce glycosuria cannot be used as a criterion. The most appropriate injection rate might be expected to lie within the normal tolerance limit at possibly 0.3 to 0.6 gm. of glucose per kilogram of body weight per hour. For a man weighing 50 kg. this would imply the injection of from 15 to 30 gm. of glucose per hour, corresponding to from 84 to 168 c.c. of 36 per cent. solution hourly, or twice these quantities of 18 per cent. solution. Although moderate accumulations of unchanged glucose in the body might do no harm or perhaps even prove desirable, it has been noted in the experiments on shock in dogs, produced by occluding the inferior cava, that a general hemorrhagic tendency supervenes when the administrations are excessive.

EXPERIMENTAL

The solutions injected consisted of chemically pure glucose in strengths of from 18 and 36 per cent., to

7. Sansum and Woodyatt (Footnote 6).

which on occasions epinephrin was added, of 0.9 per cent. sodium chlorid, and of an alkaline saline solution, 0.3 per cent. anhydrous sodium carbonate in 1.4 per cent. sodium chlorid solution (Fischer's solution). Whenever possible the physiologic sodium chlorid solution was injected either just before or just after the glucose as a control to the direct volume effects of the latter. The injections were made centrally into the vena saphena at a practically constant rate by means of an apparatus devised by Woodyatt.[8] Continuous records were made of the pressure in the carotid artery by means of a mercury manometer. Dogs were used exclusively. They were anesthetized with ether. In some experiments the rate of secretion of urine was followed, a catheter being passed into the bladder for this purpose.

Only one experiment has been done on a normal animal. The glucose solution (36 per cent.) was injected for a period of thirty minutes at the rate of 0.8 c.c. per minute (1.78 gm. of glucose per kilogram per hour). The injection raised the arterial pressure, but not more than from 5 to 7 mm. of mercury. The pulse amplitude, however, increased quite appreciably, indicating the development of some plethora. The maximum effect seems to have been attained during the first five to ten minutes of the injection, and was maintained for ten or more minutes after completion of the injection. The slightness of the rise of the pressure in comparison with the considerableness of the increase in blood volume, indicated by the change in pulse amplitude, probably is to be attributed in part to a diminution in the viscosity of the blood, due to hydremia, and in part to vasomotor accommodation.

The animal was then bled to the extent of 150 c.c., and after the pressure had become constant, well below the normal level, glucose was again injected at the same rate, but for a period of twenty minutes. During the injection period the pressure rose from 107 to 120 mm. of mercury, and during the succeeding ten minutes it continued to rise, finally reaching 125 mm. of mercury. It then declined to 111 mm. of mercury in the course of the next thirty minutes. The pulse amplitude was increased. Salt solution injected at

8. Woodyatt: Jour. Biol. Chem., 1917, **29**, 355.

the same rate raised the pressure only 5 mm. of mercury, without appreciably changing the pulse amplitude.

It is inferred from this experiment that in normal animals the compensatory mechanism tends to hold the arterial pressure close to the normal level during the plethora determined by the glucose solution; but that when, as after hemorrhage, the efforts of a normal compensatory mechanism fail to hold the pressure up, the same hydremia results in a more marked rise of pressure.

The rate of injection employed in this experiment would lead in the unanesthetized dog to a glycosuria of some 0.5 gm. per 10 kg. per hour, and this would cease within fifteen minutes after the discontinuance of the injection. In this anesthetized animal, however, the injection caused a heavy long lasting glycosuria and a coincident hyperdiuresis of such a grade that in the course of the experiment more water was excreted by way of the kidneys than was injected into the circulation.

In six experiments the effect of the injection of glucose in different doses was followed after shock had been produced by temporary partial occlusion of the inferior vena cava.[9] Our previous experience with this method of inducing shock justifies the statement that when, after deocclusion of the cava, the arterial pressure eventually begins consistently to decline, this decline proceeds, almost invariably, without break until the animal dies, though the pressure at which the decline begins may be only 20 mm. of mercury below the normal level. Despite the fact that the arterial pressure rises quite appreciably with deocclusion, we are inclined to believe that the damage that has been done to the tissues during the period of occlusion is irreversible. That is to say, despite whatever may be done, the cells of the body eventually will fail to carry on the functions necessary to the life of the organism. In such an event, the only result that could be anticipated from any form of treatment would be but a temporary improvement of certain of the functions.

Our tests of the action of glucose were begun in that stage of the experiment in which the pressure begins consistently to fall. The persistence of heavy gly-

9. Janeway and Jackson: Proc. Soc. Exper. Biol. and Med., 1915, **12**, 193.

cosuria in the normal but anesthetized dog for hours after a twenty minute injection at the rate of 1.8 gm. of glucose per kilogram per hour indicates that such a rate of infection in shocked animals which pass no urine must result in an accumulation of free sugar in the organism. The first intravenous dose at supertolerant rates must therefore give the clearest indications of what the injection of sugar is capable of accomplishing. In the table are collected the results of the first injections with the animals in shock. In evaluating the effect of the injections on the blood pressure, account must be taken of the fact that such changes as occur are built up on a pressure curve whose general tendency is to decline with time. In the construction of this table an attempt has been made to indicate this fact. For example, it is seen in the table that in Experiment 3, in which the greatest rise of pressure was recorded (from 55 to 100 mm. of mercury), the pressure at the start of the injection was practically stationary, presumably as a result of the action of salt solution injected immediately preceding the sugar injection; while Experiment 5, in which the smallest rise was recorded (from 72 to 76), was one in which the arterial pressure was falling quite rapidly at the time the glucose injection was started. The other pressure increases ranged between these extremes. On account of the difficulties just mentioned, the determination of the duration of the sugar effect on the pressure is not a simple matter. In at least two cases the maximum pressure is attained some time after the cessation of injection; and whereas in some of the other cases the maximum pressure is attained during the period of injection, the pressure curves are of such a form as to indicate that the effect is manifesting itself not alone during the whole of the injection period, but for some time subsequently.

The table also shows the effect of the sugar injection on the pulse amplitude. This is invariably increased by the injections, and the persistence of the sugar effect is brought out more clearly by the changes in pulse amplitude than by the changes in pressure. Thus in Experiment 6, whereas the mean pressure begins to fall five minutes after the cessation of injection, and in thirty minutes has returned to its previous level, the pulse amplitude at that time has not nearly declined to

RESULTS OF THE FIRST INJECTION WITH THE ANIMALS IN SHOCK

Experiment Number	Normal Mean Carotid Pressure	Pressure at Time of Injection*	Injection Period, Min.	Pressure Rise		Pulse Amplitude Increases		Dosage				Action Subsequent to Injection	Remarks
				To Mm. Hg	In Min.	From Mm.	To Mm.	Per Kg. per Hour, Gm.	Percentage Solution	Rate of Injection, C.c. per Min.	Injection Period, Min.		
1	159	51 and falling	73	69	45	1.5	3.0	0.57	18	1.0	73	The pressure is falling during the last 20 minutes of injection	Sugar injected immediately after salt solution, which had kept the pressure constant
2	140	64 and falling	68	96	68	3.5	4.5	3.7	36	1.85	68	Pressure immediately falls slowly, and, after 19 minutes, rapidly	Sugar injected immediately after salt solution, which had failed to maintain the pressure
3	117	55 and stationary	25	100++	45++	4.0	5.5	3.6	36	2.0	25	It would appear as though this animal was coming out of shock; the rise of pressure stopped only when the cava was again occluded	Immediately after 20 minutes salt injection which raised pressure 4 mm.
4	195	61 and falling	25	70	†	5.0	8.0+	2.2	36	1.0	25	.....	A previous salt injection had temporarily raised the arterial pressure 4 mm. Hg
5	142	72 and falling quite rapidly	23	76	14	6.0	6.5	3.0	36	2.0	23	.....	Pressure can be raised only by epinephrin despite the considerable increase in pulse amplitude by subsequent sugar injections
6	110	66 and falling	30	88	35	4.0	7.0+	4.0	36	2.0	30	Pressure falls rapidly after 5 minutes elapse	
8	122	78 and falling rapidly ‡	25	86	45	5.0	8.0+	3.7	36	2.0	25	Pressure falls rapidly	

* The pressure usually is uninfluenced by the sugar injection until from five to ten minutes have elapsed. This is the pressure at the moment the injection begins to cause the arterial pressure to rise.

† This was immediately followed with a stronger solution of sugar for seventeen minutes by which the pressure was raised only to 62 mm. Hg. Then epinephrin was added to the sugar. This raised the arterial pressure to 97 mm. Hg; but when the epinephrin was withdrawn, while the sugar injection was continued, the pressure fell in the course of twenty-nine minutes from 97 to 49 mm. Hg.

‡ Salt solution for twenty-five minutes did not appreciably modify the pressure curve. Ten minutes later, while the pressure was rapidly falling, sugar injection was begun. The pressure continued to fall but at a decreasing rate, and had just begun to rise at the end of the twenty-five minute period of sugar injection. As indicated, it continued to rise for twenty minutes.

its previous value. This greater persistence of the effect on the pulse amplitude than that on the arterial pressure is seen best, however, after the second and third injections.

The dosage of glucose has ranged between the extremes of 0.57 gm. per kilogram per hour and 4 gm. per kilogram per hour, that is to say, between a so-called subtolerant dose and one five times exceeding the tolerant dose. All injections resulted in a rise of pressure, which invariably was much greater than that caused by the control injection of salt solution, which often, indeed, gave an inappreciable response. Owing, however, to the difficulties mentioned, it would take a much larger series of experiments than we have performed to determine the optimum dose of glucose.

After a beginning has been made in the induction of shock by occlusion of the cava, urine secretion ceases despite the injection of sugar in doses which, in the normal animal, cause a marked diuresis. Urine was not secreted even when the alkaline hypertonic salt solution was injected.

A few tests have been made, but usually quite late in the course of the experiments, of the effect on the arterial pressure of injecting an alkaline saline solution. These tests were made only casually, and no hard and fast conclusions can be drawn from them. So far as we have gone, however, at the rate of injection employed, no obvious effects could be discerned.

We also have occasionally combined epinephrin, in concentrations ranging from 1 : 100,000 up to 1 : 25,000, with the glucose solutions. These injections also have been made late in shock. While they raised the pressure further, and possibly increased the pulse amplitude more, than the injection of simple glucose solutions, their effect was very transient and they seemed to leave the pressure at a lower level than it would have reached in the same time if nothing had been injected.

In only one case have we succeeded by the injection of sugar in turning a falling pressure into a rising pressure tending to attain the normal level. The animal in which this occurred was particularly resistant to the effects of occlusion of the cava. Whereas a two-hour partial occlusion of the cava in our experience ordinarily suffices on deocclusion to leave the arterial pres-

sure well below the normal level, and to start the pressure on a downward course within forty or fifty minutes, in this case the arterial pressure returned to the normal level after deocclusion within twenty-five minutes. After a second period of occlusion lasting forty-five minutes, the arterial pressure was still rising fifty-five minutes after deocclusion. The same was true after a third period of occlusion. It was only after the fourth period of occlusion, which lasted one and three-quarter hours, that the arterial pressure after deocclusion started on a downward course. It was then, after a preliminary test injection of saline which held the pressure practically level, that the sugar was injected. The pressure rose from 55 to 90 mm. of mercury during the injection period of twenty-five minutes, and continued to rise in the after-period, finally reaching 100 mm. of mercury, and it was still rising when, as it was believed that the animal had recovered from shock, the cava was again clamped in order again to reduce the animal to shock.

Previous experience in the production of shock indicates that when it is induced by temporary partial occlusion of the inferior vena cava, vasomotor tone, in the stage in which we have made the first sugar injections, is either below normal or at the lower limit of normal. In order to determine whether or not the difficulty in obtaining lasting improvement as a result of the injection of sugar is attributable to the damaged state of the vasomotor mechanism, in one experiment the effect of sugar injection has been tested in shock produced by temporary partial occlusion of the descending aorta. In shock thus produced, we judge from experience that the vasomotor tone, at the stage with which we are now concerned, is either normal or above normal. The experiment ran quite smoothly; but here again the action of the sugar, while quite distinct, was as ephemeral as in the majority of our experiments.

In this experiment, urine secretion stopped shortly after the aorta had been clamped, and none was formed until about two hours had elapsed after deocclusion, when, apparently under the stimulus of the sugar injection, 1 c.c. was passed. This is the only instance in which urine was formed after the production of shock in this series of experiments. Macroscopically the urine did not contain blood.

One of the most interesting phenomena brought to light by this series of experiments, and seen best after several injections have been made, is a decline of the pressure, subsequent to the injection, to a level well below that obtained previous to the injection, despite a pulse amplitude which is higher under the low pressure after the injection than it was under the higher pressure preceding the injection. The increased pulse amplitude undoubtedly is a measure of the increase in blood volume. Presumably, therefore, the pressure is lower after than before the injection, despite an increase in blood volume. This condition probably is made possible through a diminution in the vasomotor tone and a decrease in the viscosity of the blood, which together defeat the tendency of the plethora to increase the arterial pressure. It suggests that a relatively inactive vasomotor mechanism responds in the same way to its adequate stimuli as does the normal mechanism. The normal center is, so to speak, keyed to hold the arterial pressure at the normal level. The damaged center is keyed to hold the pressure at a level below the normal. And if the activity of the damaged center is constantly diminishing, the level at which it tends to hold the pressure will also constantly diminish. The reactions of the center, therefore, to increased blood volume, no matter what the new pressure normal may be, will always be such as to return the disturbed pressure to that normal. If this is the case, the combination in the late stages of shock of an increased blood volume with a lower pressure would be accounted for.

Attention should be called to the tendency exhibited by overdoses of glucose solutions to diminish the coagulability of the blood. As a result of this action, wounds which have ceased bleeding may become covered with an ooze of blood, sometimes quite considerable in amount, which remains fluid for some time. In none of our experiments was this secondary hemorrhage serious, but it is a danger which should be kept in mind when sugar injections are employed in the treatment of shock in the wounded.

SUMMARY

1. Glucose injected intravenously at rates varying between 0.57 and 4 gm. per kilogram per hour for

from twenty to sixty minutes into anesthetized dogs reduced to a state of "shock" (by partial temporary occlusions of the inferior cava or aorta) has been observed uniformly to increase the mean arterial pressure.

2. The injections have uniformly produced a marked increase in the pulse amplitude, indicating a condition of plethora.

3. The increase in pulse amplitude has usually been more striking than the increase in arterial pressure.

4. In one case the increase in pressure determined by the injection of glucose continued after the cessation of the injection until the pressure was approximating the normal.

5. A subtolerant dose has raised the arterial pressure and increased the pulse amplitude as effectively as many of the injections made at more rapid rates.

6. With the more rapid injections, a marked hemorrhagic tendency may develop in animals in this condition.

7. No other palpable deleterious effects were observed.

CONCLUSION

On theoretical and experimental grounds supported by some clinical evidence, it would appear that intravenous injections of glucose at appropriate rates are of distinct benefit in certain phases of shock.

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THE PREPARATION OF GLYCOLLIC ACID.

By Edgar J. Witzemann.

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Perhaps the simplest and most inexpensive method of obtaining glycollic acid hitherto has been the method of Hölzer[2] in which monochloroacetic acid is boiled in aqueous solution with somewhat more than the calculated quantity of calcium carbonate until no more goes into solution. The solution is then filtered and allowed to cool and crystallize. The crystals of calcium glycollate ($Ca(C_2H_3O_2)_2.4H_2O$) are washed until free from calcium chloride. Sometimes a double salt of calcium chloride and glycollate separates as a lower layer. In any case the subsequent crops are contaminated with this double salt and washing and recrystallization must be continued until all calcium chloride has been removed. These latter processes are very time-consuming. In order to obtain the free glycollic acid the calcium is precipitated quantitatively from the solution of the pure calcium glycollate with the calculated amount of oxalic acid and the solution thus obtained evaporated and crystallized. The preparation of glycollic acid may be much simplified by using barium carbonate

[1] Hofmann, *Ber.*, **17**, 1411 (1884).

[2] *Ber.*, **16**, 2955 (1883).

for the hydrolysis instead of calcium carbonate, as will be described below.

50 g. of commercial monochloroacetic acid were dissolved in 500 cc. of water. A small amount of a yellow insoluble impurity was removed by filtration and 120 g. commercial barium carbonate were added slowly. The mixture was heated on a water bath with a reflux condenser for about 30 hours, or until no more carbon dioxide bubbles were evolved. The mixture was thoroughly agitated every few hours toward the last. The excess of barium carbonate was filtered off finally and the hot solution treated with 52.1 g. 95% sulfuric acid, *i. e.*, 95% of the calculated amount necessary for complete precipitation of the barium as barium sulfate. After standing on the hot plate for some time the barium sulfate was readily filtered off on a Büchner funnel with suction. The precipitate was washed several times. The filtrate and washings were treated with small amounts of sulfuric acid until the solution showed only a slight positive test for barium. The solution was filtered again and concentrated *in vacuo*. A small amount of barium sulfate separated which was filtered off. The concentration was continued as long as water was distilled over at the bath temperature used at the beginning. The concentrated solution (a syrup) which smelled strongly of hydrochloric acid was placed on the steam bath in an evaporating dish for two hours. Not all of the hydrochloric acid was expelled in this way. The thick straw-yellow syrup weighed 40 g. (calculated yield of glycollic acid 40 g.). A drop or two of this syrup was induced to crystallize by scratching a few moments with a glass rod and this material was used to inoculate the syrup. Crystallization progressed rapidly. 16.5 g (41%) of the crystallized acid were separated in the first crop. Further crops of crystals were not obtained from this syrup. When the mother liquor was heated further on the steam plate, since it was thought necessary to remove more water in order to promote crystallization, a gelatinous anhydride separated.

The above experiment was repeated, using 100 g. of monochloroacetic acid. The almost colorless syrup in this case crystallized spontaneously. The first crop of crystals weighed 30 g. (37.5% of the calculated yield). This filtrate was heated again as above and gave a large amount of a refractive jelly which had the appearance of crystals when submerged in the syrup. This material was heated in water solution with calcium hydroxide until the solution became slightly but permanently alkaline. The first two crops of dry calcium glycollate weighed 40 g.; the third 7.8 g., the fourth 3.5, or corresponded to 41 g. of glycollic acid. The total yield of glycollic acid is accordingly 88.7% of the calculated amount, assuming that the commercial chloroacetic acid is pure.

In another experiment 200 g. of chloroacetic acid were heated on the water bath in an open flask with 1400 cc. of water and 400 g. barium car-

bonate (calculated amount 407 g.) until no more carbon dioxide was evolved. Not nearly all the carbonate had gone into solution. The mixture was diluted to about 4000 cc. finally and the heating continued. The carbonate was now completely dissolved. An excess of carbonate was now added and heating was interrupted when no more carbon dioxide was evolved. The water lost by evaporation was supplied from time to time. The barium was nearly completely removed as before and the final filtrate was treated with a pinch of washed animal charcoal to absorb the fine barium sulfate and filtered until clear. This solution was concentrated to a pale yellow syrup by evaporating on the steam bath in wide vessels. Care was taken to remove it before all water was lost. The cool syrup was inoculated and crystallized into a hard cake overnight. The mass was broken in order to permit the hydrochloric acid remaining to be evaporated. The crystal mass was broken up from time to time and finally as it became dry mashed with a pestle. After standing some days the chloride reaction with silver nitrate became weak and the odor of hydrochloric acid was absent. The crystals were dissolved in a little warm water and filtered after adding a pinch of washed animal charcoal, until the solution was clear. On inoculating the cold syrup, brilliant glass-like colorless crystals of pure glycollic acid separated in large quantities. Dried in a desiccator over sulfuric acid they melt at 78°.

The entire preparation may be carried out with almost no attention on any scale. When concentrating the aqueous solution on the water bath the evaporation must be interrupted in time or no crystals can be obtained. In case of failure to obtain crystals the syrup may be treated with excess barium hydroxide, heated until it remains permanently alkaline and treated as before, using greater care at the final stage of the evaporation.

2 g. of the pure glycollic acid were heated nine hours in a steam oven (99°). The residue weighed 1.65 g. 2 g. glycollic acid can theoretically give 1.76 g. glycollylglycollic acid, $CH_2OHCO_2CH_2CO_2H$. The viscous jelly-like syrup was treated with a little ether and then rubbed up with 25 cc. portions of water. A turbid white suspension was obtained which was diluted to 250 cc.; 25 cc. titrated with 0.1 *N* potassium hydroxide required 12.70 cc. or 127 cc. for all. This much alkali is equivalent to 1.68 g. glycollylglycollic acid. Another specimen of this thick syrup obtained from 2 g. glycollic acid weighed 1.60 g. and on standing a few days separated jelly-like turbid masses which had the same appearance under the microscope as those obtained in the crystallization of glycollic acid above, and which had no resemblance to the beautifully formed crystals of glycollic acid. It is thought that it is in part this anhydride formed so easily at 100° that prevents the crystallization of those glycollic acid syrups which were heated too long after the removal of water.

Other anhydride derivatives of glycollic acid may be formed but will not be discussed here.

It has been shown above that the method of preparation of glycollic acid is rather flexible and requires very little attention. The main advantage is that the time-consuming fractional crystallizations can be avoided by this method. It was found by experience that the method possesses no apparent advantage for the preparation of α-hydroxybutyric acid from α-bromobutyric acid.

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THE VARIATION IN THE PHYSICAL PROPERTIES OF PRECIPITATED AND COLLOIDAL MANGANESE DIOXIDE FROM THE POINT OF VIEW OF PHYSICAL CHEMICAL EQUILIBRIUM.

By Edgar J. Witzemann.

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The preparation and some striking transformations of colloidal manganese dioxide were described in a previous paper.[1] The present paper is an attempt to explain, somewhat more fully, the changes previously observed on the basis of known facts, in the hope of thereby getting a somewhat more intimate insight into the nature of certain colloidal phenomena and the equilibria involved.

In the earlier paper the history of colloidal manganese dioxide was given and the preparation and transformations were described in detail. It was found, if a glucose solution is incompletely oxidized in the cold with potassium permanganate in the presence of a little alkali, that the colloidal manganese dioxide formed under these conditions at first increases in viscosity to give a viscous or gel stage which subsequently is transformed into a limpid liquid colloidal solution. The properties of the first stage correspond well with those of an emulsoid, while the latter stage seems more characteristically a suspensoid. It was shown that the transformations of the emulsoid stage are typical and would be normal in every way if it were not for the simultaneous transformation of the emulsoid into a suspensoid. It was noticed that the latter transformation takes place more rapidly the more alkali there is present. Since A. Mayer, G. Schaeffer and E. Terroine[2] had observed that particles of negatively charged colloids tend to diminish in size in the presence of alkali and to increase in size in the presence of acid, and since colloidal manganese dioxide is a negative colloid[3] it was natural to assume that the enormous drop in viscosity here was likewise due to a great diminution in the size of the emulsoid particles on passing into the suspensoid particles under the influence of alkali. But these changes as noted by Mayer, *et al.*, were relatively small as compared with those obtained with manganese dioxide. The only experiments in which a comparable transformation was observed that could be found in the literature, were those of Scarpa,[4] in which gum arabic and gelatin emulsoids in water were transformed into typical suspensoids by the addition of definite small

[1] This Journal, **35**, 1079–91 (1915).

[2] *Compt. rend.*, **145**, 918 (1907); **146**, 484 (1908).

[3] Bredig and Marck, J. M. van Bemmelen, *Gedenkboek*, 342 (1910); for abstracts, cf. *Kolloid-Ztg.*, **8**, 333 (1911); *Chem. Abs.*, **5**, 2470.

[4] *Rend. soc. chim. ital.*, [2] **5**, 375–9 (1913); *Kolloid-Ztg.*, **15**, 8 (1914); *Chem. Abs.*, **8**, 3522.

amounts of ethyl alcohol to the cold emulsoids. It was previously known that the addition of ethyl alcohol to these solutions would cause the gum or gelatin to separate as a precipitate,[1] but Scarpa found that by using smaller amounts of alcohol the suspensoid could be obtained. That a true suspensoid was formed was indicated by the enormous fall in viscosity, by its electrophoric properties and by the difference in its behavior toward inorganic ions. Scarpa explained the transformation as follows:

"The addition of alcohol, the dehydrating properties of which are known (and which probably depend on the formation of the alcohol hydrates, which were discovered by measurements of the viscosity of its aqueous solutions), causes the removal of water from the emulsoid particles; this emulsoid must be conceived as formed of very small globules which are filled with water or a dilute solution of the substance (while their walls are composed of more concentrated solutions), or as possibly of ultramicroscopic droplets of the concentrated solution of the substance, which are suspended in the dilute solution of the same substance. In each case the result would be a contraction of the globules or droplets which finally leads to the appearance of suspensoid particles; the phenomenon would accordingly constitute a preliminary step toward the condition which according to P. P. v. Weimarn leads to the formation of the solid phase."

It is to be noted that Scarpa's transformation took place as the result of the addition of another substance to the system, while our own transformation of manganese dioxide took place as the result of compounds already present in the mixture, *i. e.*, represented the establishment of an equilibrium between the compounds that took part in the reaction. It will be shown below that this difference is not so significant as it at first seems.

Since Scarpa's explanation hinges on the dehydrating effect of ethyl alcohol, the question at once arises as to what agency in our own case produces this effect on the manganese dioxide colloid. Recently G. Poma and A. Patroni[2] studied the influence of neutral salts on the conditions of ions in solution. They used solutions of copper nitrate in the presence of nitrates of Rb, K, Na, Li, Sr, Ca and Mg as well as ethyl alcohol, in order to study the displacement of the equilibrium expressed thus

$$[Cu''] \rightleftarrows K[Cu(H_2O)''_n]$$

due to the presence of these compounds. If $[Cu''_o]$ represents the concentration of the Cu ion in the absence of neutral salts and $[Cu'']$ represents the concentration in the presence of neutral salts, then the addition of nitrates of K, Rb, Na and Li were found to increase the ratio

$$[Cu''_o] \quad : \quad [Cu''],$$

i. e., the concentration of Cu ions is increased by the presence of these salts. It was noted that the nitrates crystallizing with the greatest amount of water of crystallization produce the largest effect, and that the effect of the neutral salt is to displace the equilibrium

$$[Cu(H_2O)''_n] \rightleftarrows Cu'' + nH_2O$$

[1] P. P. v. Weimarn, "Grundzüge d. Dispersoidchemie," p. 116; *Kolloid-Ztg.*, **2**, 78 (1907).

[2] *Z. physik. Chem.*, **87**, 196–214 (1914); *Chem. Abs.*, **8**, 2094.

to the right. If the above assumption is tenable Poma and Albonico[1] considered that the analogous effect should be observed in the hydrolysis and esterification equilibria of esters in the presence of a strong acid and of a neutral salt of the above type. Without attempting to report their results in full, one of the general conclusions drawn by them from their experiments was that the presence of a neutral salt changes the homogeneous equilibrium of esterification and hydrolysis as if part of the water present in the system were removed from an active role in the immediate equilibrium. This displacement depends on the concentration of the neutral salts used and on the nature of their cations; it increases rapidly with the diminution of the electroaffinity of the latter and thus the effect increases in the order K, Na, Li, Ca and Mg. As a whole the results may be completely explained by assuming that the neutral salts in solution form hydrates, the complexity of which increases with the decrease in the electroaffinity of their ions.

The work cited above shows rather conclusively that the hydration-dehydration phenomena in the case of ionizable crystalloids are true equilibria and are amenable to the laws that have been found to apply to chemical equilibria. If the general results described above are true for crystalloids there is no *a priori* reason why the same general results should not be true for colloids. As a matter of fact Galeotti, in 1904,[2] examined the precipitation of metallic compounds of the proteins and found that the composition of the precipitate depends on the composition of the solution with which it is in contact. From his results he was able to construct the triangular diagram for the ternary system: metallic salt–protein–water. He therefore concluded that this system obeys the thermodynamic laws of chemical equilibrium. From a study of the phase rule diagram for the ternary system: egg albumin–ammonium sulfate–water, the same conclusion was arrived at. Galeotti did not, however, consider the chemical and physical forces involved in these equilibria since he was primarily interested in establishing the application of the phase rule to these phenomena.[3]

[1] *Atti accad. Lincei*, **24**, I, 747–54, 979–85; II, 43–51 (1915).

[2] "Über die sogenannten Metalverbindungen der Eiweisskörper nach der Theorie der Chemischen Gleichgewichte," *Z. physiol. Chem.*, **40**, 492–547 (1904); "Über die Gleichgewichte zwischen Eiweisskörper und Elektrolyten. I. Eieralbumin, $(NH_4)_2SO_4$-H_2O," *Ibid.*, **44**, 461 (1905).

[3] No attempt has been made to determine who first thought of these processes of equilibria. A few instances in which the idea was most clearly stated were finally found and have been selected for a historical basis. The fact that none of the authors cited had found an earlier statement of the principle indicates how little emphasis has been placed on the matter. It seems that our widely accepted theories of osmosis and electrolytic dissociation, which are admittedly only accurately applicable to certain kinds of weak solutions, prevent us from thinking clearly about phenomena in intermediate and concentrated solutions.

In looking for facts that would assist in elucidating these phenomena it is found at once that another group of workers in studying the action of neutral salts and other compounds on emulsoids have found that the viscosity of emulsoids is diminished in many cases by the addition of these compounds. This diminution in viscosity is commonly ascribed to dehydration of the colloid particles and in the case of electronegative colloids the effect increases with the diminishing electroaffinity of the cations, as was noted in the equilibria studied by Poma and Albonico.

Now it seemed a simple matter to assume that the deductions of Galeotti for the three component heterogenous aqueous protein systems are applicable to colloids in general and that one of the factors in what are generally acknowledged to be complex equilibria (cf. Poma, *et al.*) is the hydration $\rightleftarrows$ dehydration equilibrium. It was of interest to apply this conception to the colloidal manganese dioxide system. It was also important to learn whether the existence of other equilibria could be deduced from the experimental observations.

Experiments with Acetone.

Since the role of glucose as a colloid protector in the preparation of colloidal manganese dioxide was not understood, a series of experiments was carried out in which acetone was used instead of glucose. In this series 2 g. of pure acetone were diluted to 1 liter with water containing potassium hydroxide, as follows:

(1) 0. g. KOH	(5) 4.86 g. KOH	(9) 9.72 g. KOH	(13) 19.55 g. KOH
(2) 1.2 g. KOH	(6) 6.06 g. KOH	(10) 10.92 g. KOH	(14) 38.88 g. KOH
(3) 2.43 g. KOH	(7) 7.29 g. KOH	(11) 12.15 g. KOH	(15) 77.76 g. KOH
(4) 3.63 g. KOH	(8) 8.49 g. KOH	(12) 14.58 g. KOH	(16) 155.52 g. KOH

A small amount (about 0.2 g.) potassium permanganate was added to each bottle and the mixture agitated vigorously for about 15 seconds. The reduction to green potassium permanganate was more prompt the greater the potassium hydroxide concentration. The contents of bottle number (1) showed no reduction at all after standing 18 hours. In all of the others the character of the manganese dioxide precipitate after standing 18 hours showed an interesting variation in the various experiments. The precipitates in numbers (2), (3), (4) and (5) were very voluminous and in the remainder the volume gradually diminished until in the last three or four experiments of the series it was relatively small.

At the end of the second day, after having added the above amount of permanganate three times, the experiments stood as follows: (1) unreduced; (2) and (3) very dark brown flocculent manganese dioxide precipitate one inch deep; the precipitates in the remainder are similar except that the thickness of the precipitate layer is progressively thinner as the concentration of the alkali increases. This progressive diminution in the thickness of the precipitated layer is evident in number (6) and becomes more

and more marked on passing gradually to number (16). After agitating the mixtures it was noted that the ones having the higher alkali content settled most quickly. However, in the last four or five experiments of the series only the upper one half of the liquid is quite clear after standing quietly for a few hours, showing that there are some very fine particles present in these cases.

After the addition of potassium permanganate had been continued for 9 days it became increasingly evident that with age the marked differences in the character of the precipitates was gradually diminishing. During the first few days the new portion of manganese dioxide precipitate floated on top of the older portion of the precipitate as a thick flocculent layer, but this difference in the character of the older and new portions of the precipitate diminished as the experiments were continued, owing perhaps to changes in the composition of the solution or to the increasing bulk of the precipitate.

After standing for nine months there was scarcely any perceptible difference in the physical character of the precipitated manganese dioxide. This would indicate that in this case a small amount of alkali acting for a long time has about the same effect as a larger amount acting for a short time.[1]

From the above it is clear that although in this case colloidal manganese dioxide was not obtained in the emulsoid-suspensoid form, the amorphous form, which is truly colloidal according to J. M. van Bemmelen,[2] shows variations in its physical characteristics which are strictly comparable and analogous to the emulsoid-suspensoid stages.

In thinking of the possible explanations of the variations in the physical character of the precipitated or amorphous colloidal manganese dioxide it seems that there are two possibilities: (1) the differences in the precipitate are due to differences in the size of particles; (2) the differences are due to variations in the density of the particles. If (1) is correct then on long standing the fine particles would finally settle down to nearly the same extent as the coarse ones since they would be capable of being packed together even more densely than the larger ones. But on standing many hours the supernatant liquid in the solutions of low alkali content is clear, and the precipitated layer is several times as thick as in the experiments containing more alkali. Since the particles in the more alkaline experiments are obviously finer, these precipitates should be the slowest to settle. The contrary was observed. Thus Explanation 1 does not hold. In considering the second explanation it must be recalled that a chemical

[1] Observations, on precipitated manganese dioxide, similar to those described above were made in the oxidation of glucose with potassium permanganate, in the presence of variable amounts of alkali (THIS JOURNAL, 36, 160–6 (1916)).

[2] "Die Absorption," p. 90 (1910).

compound will have the same density whatever the size of its particles, so that *a priori* Explanation 2 only becomes possible if it is assumed that something has combined to a variable extent with the manganese dioxide precipitate. Since the alkali content of the precipitated manganese dioxide seems to be rather constant, whereas the water content is known to be quite variable, it is simple to assume that the amount of water associated with the manganese dioxide is indeed variable. Since water has a much smaller density than manganese dioxide, those precipitates containing the most combined or adsorbed water would settle more and more slowly the more nearly their density approached that of water. At the same time the bulk of the precipitate would be expected, as was observed, to increase owing to the presence of the water. Explanation 2 therefore coincides with the observed facts.

As was noted above, the character of the precipitates had not assumed a definite form until some hours had elapsed. We are therefore warranted in assuming that the initial state from which the observed state arose was closely similar in each case. That is, if we apply the principle of neutral salt effect as observed by Poma, *et al.*, and started with hydrated manganese dioxide in the presence of variable amounts of alkali, the rate at which it would be dehydrated would in accordance with the law of mass action increase with increase in the concentration of the alkali. This is in accordance with the observed facts. Thus it is evident that so far as alkali concentration is concerned the dehydration of amorphous precipitated manganese dioxide is a true equilibrium.

The difficulty with the conclusion drawn in the preceding paragraph is that it is based on an assumption concerning the conditions in the beginning of the transformation that could not be followed by direct observation. If we turn now to the observations made with the emulsoid-suspensoid form of colloidal manganese dioxide (*loc. cit.*) we can follow the early stages of the transformation of the potassium permanganate much more clearly.

In any aqueous colloidal system containing a neutral salt, there are, it seems, besides the ionic equilibria at least seven types of molecular equilibria: three molecular association $\rightleftarrows$ molecular dissociation and three hydration $\rightleftarrows$ dehydration equilibria and one "adsorption equilibrium" as follows:

(1) $x\mathrm{H_2O} \rightleftarrows (\mathrm{H_2O})_x$
(2) x salt $\rightleftarrows$ $(\mathrm{salt})_x$
(3) x Colloid $\rightleftarrows$ $(\mathrm{Colloid})_x$
(4) $[x \text{ salt} \rightleftarrows (\mathrm{salt})_x] \rightleftarrows [x\mathrm{H_2O} \rightleftarrows (\mathrm{H_2O})_x]$
(5) $[x \text{ Colloid} \rightleftarrows (\mathrm{Colloid})_x] \rightleftarrows [v\mathrm{H_2O} \rightleftarrows (\mathrm{H_2O})_x]$
(6) $[x \text{ salt} \rightleftarrows (\mathrm{salt})_x] \rightleftarrows [x \text{ Colloid} \rightleftarrows (\mathrm{Colloid})_x]$
(7) Equilibrium 4 $\rightleftarrows$ Equilibrium 5

That is, in (1), (2) and (3) the simple molecules are in equilibrium with their polymers. In (4) the salt equilibrium is in equilibrium with the water equilibrium. The product in this case would be the salt hydrates. In (5) the colloid equilibrium is in equilibrium with the water equilibrium. The product in this case would be the colloid hydrates. In (6) the salt equilibrium is in equilibrium with the colloid equilibrium. The product in this case would be the "absorption compounds." In (7) we have the hydrated form of "Equilibrium 6, *i. e.*, the salt-hydrate Equilibrium 4 in equilibrium with the colloid-hydrate Equilibrium 5. The result in this case is the establishment of equilibrium among the products of Equilibria 4, 5 and 6.[1]

In the case of the emulsoid-suspensoid manganese dioxide we started with prepared solutions of potassium permanganate and glucose containing some alkali. In each solution the water and the solutes (*i. e.*, Equilibria 1, 2 and 3) were presumably in equilibrium. When, however, the potassium permanganate solution was gradually added to the glucose solution in the cold a reaction took place which resulted in the reduction of permanganate to manganese dioxide. We now have the additional association Equilibrium 3, since manganese dioxide is colloidal. Although the strong color of permanganate solutions has been ascribed to complex associated molecules, the polymeric tendencies become relatively enormous in manganese dioxide, so that we can observe the color changes after the permanganate color is all gone and the enormous increase in viscosity during the short interval following the reduction. That this change is due to polymerization is indicated by the corresponding changes in color and viscosity in homogeneous systems such as styrol, isoprene, etc. Since the energy balance is so favorable to this polymerization, as indicated by its great velocity, the reasonable expectation is that the viscosity will continue to increase, as is noted in the case of styrol and isoprene. But this was not the case. The viscosity increases up to a certain maximum and what happens subsequently depends in part on the initial concentration of the alkali. If sufficient or excess alkali was present the gel begins to "melt" more or less quickly, depending on the concentration of the alkali and on the temperature.

At the beginning the potassium permanganate and its ions are in equilibrium with the water in which they exist. The manganese dioxide to which it is reduced in the reaction[2] has a strong affinity for water and no doubt tends to inherit the water of the permanganate from which it is

[1] The equilibria have all been written in the molecular form. In our own case this seems simpler and so far quite satisfactory. But it seems certain that in many colloids the equilibria for the ions would be very significant. There are a number of other interesting considerations which could have been drawn into the discussion but which have been reserved for the discussion of other experiments.

[2] van Bemmelen, *Die Absorption*, p. 90 (1910).

derived. The potassium salts have a strong affinity for water likewise. We have thus set up a new equilibrium. An examination of our seven equilibria shows that the one that is most significant in this latter stage is the salt-hydrate $\rightleftarrows$ colloid-hydrate Equilibrium 7. Since the experimental result in this case is the transformation of the emulsoid into the suspensoid it is clear that the equilibrium is in favor of the salt-hydrate side and we again have the dehydration discussed above for the amorphous colloidal manganese dioxide taking place.

In the earlier paper it was stated that "All the properties of colloidal manganese dioxide may therefore be explained by the simultaneous action of two forces: the one tending to condense and precipitate the colloid and the other tending to disperse the colloid. The former is a very rapid-acting process and is hastened by increase in temperature and absence of alkali. The latter is a slow-acting process in any case, but can be accelerated greatly by relatively small increases in the alkali concentration and less markedly by increase in temperature up to a certain rather low maximum." From the above it would seem that the first force mentioned is the mutual affinity of the manganese dioxide molecules tending to polymerize the manganese dioxide up to a highly complex molecular aggregate and therefore involves Equilibrium 3. The second force mentioned is a much feebler, therefore more slow-acting force, which controls the equilibria of the dehydration of the manganese dioxide aggregates, *i. e.*, Equilibrium 7. The relative greatness of the first force, as compared with what is observed in other colloids ordinarily, explains why manganese dioxide is so frequently found in the amorphous colloidal form described above instead of the emulsoid-suspensoid form. Moreover, it seems simple to assume that the colloid protecting action of glucose consists in preventing the association Equilibrium 3 from completing the polymerization by virtue possibly simply of mechanical interference. Thus if glucose is substituted for salt in Equilibrium 6 its protective action may consist in the formation of an "adsorption compound." At the same time the free glucose by virtue of its dehydrating power favors the result obtained from Equilibrium 7. Thus if we conceive of the energies involved in these changes as composed of an intensity and a capacity factor it is clear that the intensity factor of the first force involved is very favorable, while it is the capacity factor that is most significant in the second force.

Summary.

It has been previously found that colloidal manganese dioxide prepared from aqueous solutions of glucose and potassium permangante in the presence of some alkali underwent colloidal transformations which varied strikingly with the conditions and which seemed to be definitely dependent on equilibria, the nature of which was not known. It has now been shown

that the amorphous colloidal manganese dioxide obtained by reducing permanganate with acetone in the presence of variable amounts of alkali undergoes changes comparable with those of the emulsoid-suspensoid type of manganese dioxide. On the basis of certain results of other workers the attempt was made to interpret the experimental data for both types of colloids on the basis of two types of dynamic equilibria: (1) molecular association $\rightleftharpoons$ molecular dissociation; (2) hydration $\rightleftharpoons$ dehydration. It was found that the experimental observations readily fit into such an interpretation.

It is thought that the point of view developed here will be of much interest in the elucidation of a number of other chemical and biological phenomena and further work along these lines is under way.

CHICAGO, ILL.

[Contribution from the Otho S. A. Sprague Memorial Institute, Laboratory of Clinical Research, Rush Medical College.]

THE ROLE OF ATMOSPHERIC OXYGEN AND ALKALI IN THE OXIDATION OF ACETONE WITH POTASSIUM PERMANGANATE. PYRUVIC ACID AS AN INTERMEDIATE PRODUCT.

By Edgar J. Witzemann.

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Since our knowledge of the nature of the chemical equilibria active in oxidations is quite limited it is highly desirable that more work be done to extend this knowledge. The following paper deals with the oxidation of acetone with potassium permanganate in the presence of varying amounts of alkali at room temperature. The purpose of these experiments was fourfold:

(1) To study the relative participation of atmospheric oxygen in the oxidation of acetone.

(2) To study the relative yields of acetic, oxalic and carbonic acids and to note, if possible, the reasons for the variations.

(3) To form a general explanation of the mechanism of the oxidation of acetone in alkaline solutions.

(4) To get first-hand accurate knowledge concerning the relative ease of oxidation of acetone and glucose.

In some previous experiments on the oxidation of glucose[1] with potassium permanganate it was noticed, when the initial concentration of alkali was varied, that among other things the amount of participation of atmospheric oxygen in the oxidation varied. Since it was well known that glucose in alkaline solution is oxidized by air and since it was likewise known that manganese oxide (MnO) is sensitive to air oxidation especially in the presence of alkali there were three possible explanations for the participation of atmospheric oxygen in this oxidation.

(1) The atmospheric oxygen in the presence of alkali acts directly upon the glucose.

(2) The atmospheric oxygen in the presence of alkali acts upon the manganous oxide to form manganese dioxide, which, in turn, is reduced again by the glucose.

(3) The simultaneous action of (1) and (2).

In order to learn to what extent (2) might be significant it was desirable to oxidize some compound less sensitive than glucose to atmospheric oxidation in order to see if the same phenomenon occurred. Acetone is a compound that is well adapted to this purpose. In some experiments to be described it was shown that acetone is not acted upon by atmospheric oxygen even in the presence of alkali. In fact acetone is difficultly oxidized even by potassium permanganate as is indicated by the fact that the organic chemist has long been accustomed to use acetone as the solvent in the permanganate oxidation of organic compounds not sufficiently soluble in water,[2] because it is so little acted upon by the permanganate. The results show that the above considerations do not rule out the large participation of atmospheric oxygen in these oxidations.

The second reason for doing these experiments was to learn more about the oxidation of acetone with permanganate. It was especially desired to know more about the effect of changing conditions on the nature of the products of oxidation. Hercz,[3] in studying the behavior of various ketones with respect to Popoff's rule, found that acetone oxidized with two atoms of oxygen as potassium permanganate[4] for each molecule of acetone gave only acetic, carbonic and traces of formic acids. G. Wagner[5] alleged that the course of such oxidations was independent of the nature

[1] This Journal, 38, 150 (1916).

[2] Sachs, *Ber.*, 34, 497 (1901).

[3] *Ann.*, 186, 257 (1877).

[4] [Cochenhausen showed later (*J. prakt. Chem.*, 58, 451 (1898)) that more than two atoms of oxygen are required.]

[5] *Ber.*, 25, Ref. 121.

of the oxidizing agent. Cochenhausen,[1] by using the potassium permanganate is variable but up to excessive proportions in very strongly alkaline solutions, found from 78.76% to 85.39% of the calculated amount of oxalic acid when using from 2.5 to 20 atoms of oxygen per molecule of acetone when the results were calculated according to the reaction

$$3CH_3COCH_3 + 21O \longrightarrow 3(CO_2H)_2.2H_2O + 3CO_2. \qquad (1)$$

The alternate reaction

$$3CH_3COCH_3 + 14O \longrightarrow 2(CO_2H)_2.2H_2O + 2CH_3CO_2H + 2CO_2 \qquad (2)$$

is, according to Cochenhausen, only of significance when quite insufficient permanganate is used, but accounts for the acetic acid found in those cases. In no case did Cochenhausen study the effect on the relative yield of acetic and oxalic acids, of varying the alkali concentration in his oxidation. Since he did not determine how much acetic acid was formed, these data cannot be obtained from his results. He states that he used 10 g. potassium hydroxide in each experiment and that he diluted the reaction mixture to 200 cc. before analyzing but does not state what the concentration was at the time when the acetone was added.

Denis[2] studied the action of potassium permanganate on acetone in the course of more general study of the mechanism of oxidation. She performed three oxidations of acetone and found that acetic acid is formed as well as carbonic and oxalic acids and that the relative amount of oxalic acid increases with the concentration of potassium hydroxide used. These data are, however, not sufficient for our purposes.

The third reason for these experiments was to get some additional data for the oxidation of acetone in alkaline solutions. The existing amount of careful quantitative work on the oxidation of aliphatic compounds is still altogether too small to permit of accurate and definite applications of the results to the biological oxidation of fats and sugars.

The fourth reason for these experiments was of the greatest interest to the author. In a previous paper[3] the oxidation of glucose with potassium permanganate in the presence of variable concentrations of alkali was studied with great care. It was now of interest to study the conduct of acetone under the same conditions. In certain diseases, and in fasting and starvation, it is generally thought there is a tendency for the fats to undergo the so-called β-oxidation, in part at least. The end members of this process are in part acetoacetic acid and acetone into which it spontaneously decomposes. When glucose (starch or sugar) is adequately metabolized by these individuals the excretion of these so-called "acetone bodies" ceases. It has never been adequately shown whether the glu-

[1] *J. prakt. Chem.*, **58**, 451 (1898).
[2] *Am. Chem. J.*, **38**, 572 (1907).
[3] *Loc. cit.*

cose facilitates the oxidation of these compounds, as maintained by some or whether it prevents their formation. The author is of the latter opinion. These experiments offer some basis for this opinion and make a quantitative study of the oxidation of glucose and acetone within the same solution possible.

Experimental Part.

All the oxidations were carried out by dissolving 2 g. of pure acetone in 1000 cc. of water containing variable amounts of potassium hydroxide as shown in the tables. Two series of experiments were carried out owing to the fact that certain irregularities occurred in the first series. The two series amplify and confirm each other. The details of procedure were the same as in the oxidation of glucose.[1] Small amounts of powdered permanganate were added to each flask and the mixture agitated from time to time until reduction was complete. When reduction no longer took place even after standing for several days the oxidation was considered complete. In Expt. 3, in Series II, reduction must not have been as complete as was thought when the oxidation was stopped. Details about the transformations of colloidal manganese dioxide in the first stages of the oxidation of acetone in this way have previously been published.[2]

The analytical methods were the same as those previously described.[3] No method for determining the small amount of acetic acid was found that was entirely satisfactory. In some instances it was determined in the presence of hydrochloric acid;[4] sometimes by the phosphoric acid method by distilling *in vacuo* or at ordinary pressure. Distillation with phosphoric acid *in vacuo* was in general most satisfactory.

Discussion of the Results.

Potassium Permanganate.—As stated in the introductory part one purpose of this work was to continue the observations on the relative participation of atmospheric oxygen in oxidations in alkaline solutions with potassium permanganate. In the first place it was necessary to know whether acetone is oxidized by atmospheric oxygen. In order to determine this 1 g. acetone was made up to 500 cc. with enough alkali to make the concentrations as follows: (1) no alkali; (2) 0.01 N potassium hydroxide; (3) 0.05 N; (4) 0.010 N; (5) 0.20 N. 5 cc. of the mixture were titrated at once for acetone by the Lieben iodoform method. 1.04 g. was the average acetone content. The flasks were stoppered tightly with rubber stoppers and were agitated vigorously at least once each day. After 12 days at room temperature the average acetone content was

[1] *Loc. cit.*

[2] This Journal, **39**, 28 (1917).

[3] *Loc. cit.*, p. 152.

[4] Evans and Witzemann, This Journal, **34**, 1086 (1912).

1.03 g. After 1 month the average acetone content was found to be 1.03 g. These experiments show that acetone is not oxidized by atmospheric oxygen.

For details concerning the interpretation of the permanganate deficiency see the earlier paper.[1] It is sufficient to say here that a comparison of the values in Cols. II and III in the tables shows that here too there is a deficit in the amount of permanganate actually utilized as compared with that calculated from the products of the reaction. In this case the deficit is more regular throughout the series and in neither series does it reach such high values as with glucose. The irregular values are in part due to the irregularities in the analysis of the products of the oxidation (especially acetic acid) from which these deficits were derived by calculation.

In general, we may conclude that since atmospheric oxygen does not act upon acetone in alkaline solutions up to 0.20 *N* concentrations, that if atmospheric oxygen does directly oxidize acetone according to the first suggested explanation, it does so at some stage lower in the oxidation than acetone itself.

In the second series of oxidations which were carried out more quickly the permanganate deficits were smaller in the less alkaline solutions than in the first series.

Products of the Oxidation.—In both series oxidation in the first experiment was imperceptibly slow. On standing a month or more the first small portion of permanganate (0.1–0.2 g.) added was partially reduced.[2]

In Expts. 2 and 3 in both series the oxidation was more rapid but came to equilibrium before all the acetone was reduced, as is shown by the figures in Col. XVII. The oxidation in Expt. 3, in Series II, was probably incomplete. In the second series the unchanged acetone was determined in 13 experiments and the results showed that in the remaining experiments oxidation was complete.

The general results showed that it is possible to obtain every degree of velocity of oxidation from zero to almost instantaneous reaction in the oxidation of acetone with potassium permanganate by suitable variations in the alkalinity. It is thus clear that in these experiments the alkalinity alone determines the velocity of oxidation.

Cochenhausen[3] concluded from his study of this reaction that in the presence of alkali and sufficient permanganate the main reaction is

[1] *Loc. cit.*, pp. 155–8.

[2] It has long been known that acetone is resistant to oxidation by even such a powerful oxidizing agent as potassium permanganate. Thus Péan de Saint-Gilles (*Compt. rend.*, **47**, 554 (1858)); *Ann. chim. phys.*, [3] **55**, 374 (1858)) as well as Sachs (*Ber.*, **34**, 497 (1901)) knew that permanganate is dissolved unchanged in acetone. In fact permanganate has been used to remove methyl alcohol from commercial acetone (Friedel, *Ann. chim. phys.*, [4] **16**, 405 (1869); Martine, *Ibid.*, [8] **3**, 82 (1904)).

[3] *Loc. cit.*

(1) $3CH_3COCH_3 + 21O \longrightarrow 3(CO_2H)_2.2H_2O + 3CO_2$

and that

(2) $3CH_3COCH_3 + 14O \longrightarrow 2(CO_2H)_2.2H_2O + 2CH_3CO_2H + 2CO_2$

is only of significance when inadequate amounts of permanganate are used. The latter reaction was designed to explain the formation of acetic acid and in doing so apparently assumes that some sort of a condensation takes place since otherwise three molecules of carbon dioxide and one or two molecules of acetic or oxalic acid would have been formed. The results of my experiments show that Reaction 2 is not true at least for the conditions used in these experiments.

The tabulated results and the earlier work cited throughout this paper indicate clearly that in the formation of the final products there are at least two courses of reaction:

(1) $CH_3COCH_3 + 4O \longrightarrow CH_3CO_2H + CO_2 + H_2O$

(2) $CH_3COCH_3 + 7O \longrightarrow (CO_2H)_2 + CO_2 + H_2O,$

occurring simultaneously and the extent to which the one or the other preponderates depends on the velocity of that reaction under the conditions of the experiment. Of these the second is identical with Cochenhausen's first reaction. The results show that the first reaction becomes less significant as the alkalinity increases so that in Expt. 16 the oxidation takes place about 65% according to Reaction 2 and only about 35% according to 1. The results also show that very little, if any, acetone is oxidized solely to carbon dioxide thus:

$$CH_3COCH_3 + 4O_2 \longrightarrow 3CO_2 + 3H_2O$$

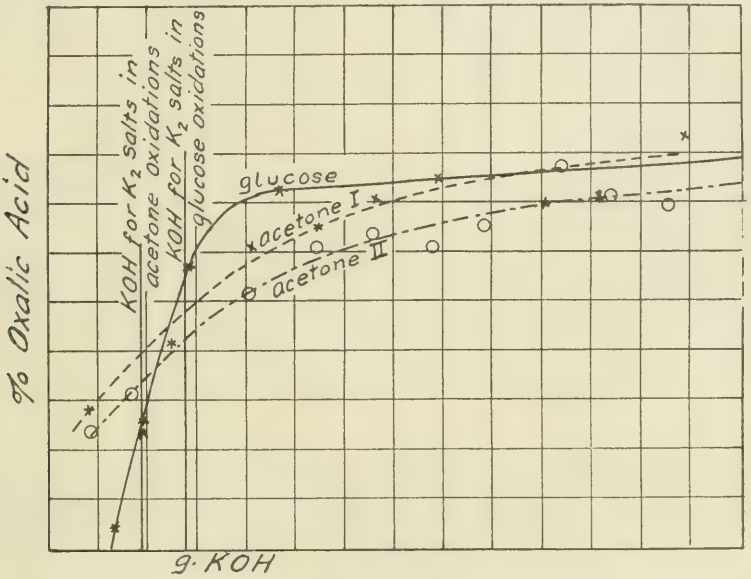
Much of the discussion of the products of oxidation in the paper on glucose oxidation[1] is of interest in this connection, but will not be repeated here. One of the most interesting facts brought out in that discussion was the fact that an additional 0.8 g. of potassium hydroxide in one experiment as compared with the preceding caused an increase of 10.6% in the yield of oxalic acid. Calculations showed that this sharp change in the nature of the products was due to the fact that when the available alkali becomes equal to the amount required for normal salts of the products the yield of oxalic acid becomes maximum (see the graph). Owing to the fact that oxalic acid in alkaline solutions is not freely oxidized, the results indicated that one important role of the alkali was to stabilize the oxalic acid which is also an intermediate in the oxidation of glucose even in the absence of alkali.[2]

In the case of the acetone oxidations the available alkali (Col. XXI) exceeds that required for the formation of normal salts in Expts. 3 to 16, but there is no such break in the yield of oxalic acid as was observed in

[1] *Loc. cit.*, pp. 158–60.

[2] Smolka, *Sitzb. Math. nat. Klasse k. Akad. Wiss., Wien*, **2**, 5–30 (1887).

the oxidation of glucose. In fact the yield in oxalic acid rises gradually and continually from the first to the last experiment (see the graph). This would indicate that there is an intermediate rearrangement or transformation upon which the formation of oxalic acid is dependent. When this transformation does not take place the intermediate compound in



question is oxidized to acetic and carbonic acids instead of oxalic and carbonic acids. The extent to which this transformation takes place is indicated by the ratio of the two groups of products since both are stable toward permanganate under the conditions of the experiment.

Such an intermediate change could be the enolization of pyruvic acid, $CH_3.CO.COOH$ into $CH_2 : COH.COOH$, discussed below.

The Mechanism of the Oxidation.

In the earlier experiments on the oxidation of glucose under analogous conditions very little could be said about the mechanism of the oxidation from the point of view of the glucose. It seems much more likely that the mechanism of the oxidation of acetone can be more fully interpreted owing to the relative simplicity of its constitution. Dr. Denis[1] has in fact already discussed the mechanism of this process rather fully on the basis of Nef's conceptions and came to the conclusion that the formation of oxalic acid from acetone is due to the successive intermediate formation of acetol, pyruvic and mesoxalic acids. The acetol, in her opinion, is obviously obtained from the oxidation of isoacetone molecules, which are present in an alkaline solution of acetone. Her argument for this conception is summarized in the following quotation:

[1] *Am. Chem. J.*, **38**, 561 (1907).

"An aqueous solution of acetone contains the following molecules in dynamic equilibrium:

$$CH_3COCH_3 \rightleftarrows CH_3COH + \diagup\!\!\diagdown CH_2$$

"When therefore, this ketone is oxidized in neutral or acid solution with an excess of permanganate, bichromate, etc., acetic and carbonic acids are formed in exactly molecular amounts. An aqueous solution of acetone can consequently contain no trace of enol or β-hydroxypropylene molecules. As soon however, as a certain definite and very small amount of potassium hydroxide is added to the solution the presence of isoacetone molecules can at once be rigidly established by experimentation in three different ways:

"(1) By the quantitative transformation into mercuric-β-hydroxypropylene.

"(2) By conversion of acetone into diacetone alcohol, $(CH_3)_2:COH.CO.CH_3$ (addition of the β-hydroxypropylene molecules, with anionic rearrangement, to unchanged acetone).

"(3) By the almost quantitative oxidation of the β-hydroxypropylene molecules with intermediate formation of acetol, trihydroxypropene, $CH_3C(OH)_2CHOH$, into equimolecular quantities of carbonic and oxalic acids.

"Acetone, as has long been known, does not react in aqueous solution with freshly precipitated and well washed mercuric oxide. As soon, however as an alkali is added a practically quantitative conversion into white mercuric isoacetone, $(CH_3.CO.)_2Hg$, $\|$ CH_2 takes place with marked evolution of heat.

"This shows very clearly (1) that an aqueous solution of acetone contains no β-hydroxypropylene molecules; and (2) that such molecules appear in the solution of addition of small amounts of potassium or sodium hydroxide and that their presence can at once be recognized in the three ways indicated above."

Before fully accepting the above interpretation of the behavior of acetone it is desirable to see whether it is in harmony with other observations. The only other oxidation of acetone that is quantitatively carried out in an alkaline medium is the titration of acetone as iodoform by the Lieben method. The most adequate existing explanation of this oxidation is that of Pieroni and Tonnioli[1] and involves a conception fundamentally similar to that of Denis.

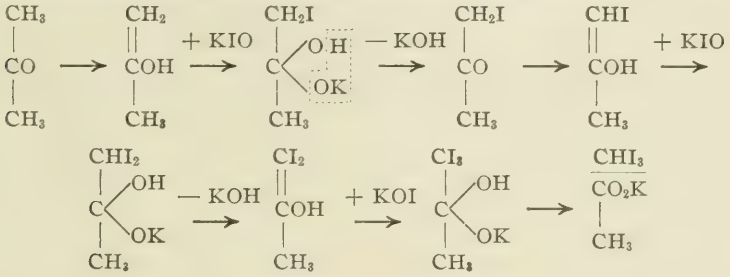
Pieroni and Tonnioli found in a study of the mechanism of the formation of iodoform from acetone and acetaldehyde[2] that the formation of iodoform is due to the action of hypoiodite and the quantity of iodoform produced depends on the quantity of hypoiodite that reacts. In order to explain the fact that 93.4% of the iodine consumed is combined in iodoform instead of 50% as required by an older theory, on the assumption that half of the iodine is lost as hydriodic acid Pieroni and Tonnioli formulated an explanation based on the fact that ethylene adds hypochlorous acid to form glycolhydrin

$$CH_2 : CH_2 + HClO \longrightarrow CH_2OH.CH_2Cl.$$

[1] *Gazz. chim. ital.*, **43**, II, 620 (1913).

[2] *Ibid.*, **42**, I, 534 (1912).

They represent their explanation graphically as follows:



Since the reaction as far as the iodine is concerned is 93% quantitative it follows that the deviations from the reaction, according to which one molecule of acetone gives one molecule of iodoform and one of acetic acid, cannot be great. So that although there is room for side reactions, they must be of relatively small importance. If we accept Denis' first suggestion about the dissociation of acetone thus:

$$CH_3COCH_3 \rightleftarrows CH_3COH + \diagup\!\diagdown CH_2,$$

we should expect the acetaldehyde to react with hypoiodous acid to give iodoform finally[1] and likewise the methylene residue should react. This would result in the utilization of more iodine by this acetone molecule than is provided for on the basis of the above reaction scheme. Thus it is possible that the discrepancy of 7% may be accounted for by just such a side reaction as that provided for by the above-mentioned dissociation equilibrium.

Thus by assuming that dissociations of the above type exist in acetone solutions as well as the isoacetone equilibrium (enol form) it becomes possible to understand both types of reactions in the same medium by remembering that the velocity constants for the two types must be quite different.

About a year after the appearance of Denis' paper Fournier[2] studied the oxidation of acetone with potassium permanganate in the presence of sodium, potassium, calcium and barium hydroxides as well as sodium and potassium carbonates with special reference to the intermediate formation of pyruvic acid. The pyruvic acid was isolated as the phenylhydrazone and although the reaction was very sensitive to slight changes in conditions, the maximum yield was 9% pyruvic acid as the phenylhydrazone. Glückmann had previously[3] obtained a similar result.

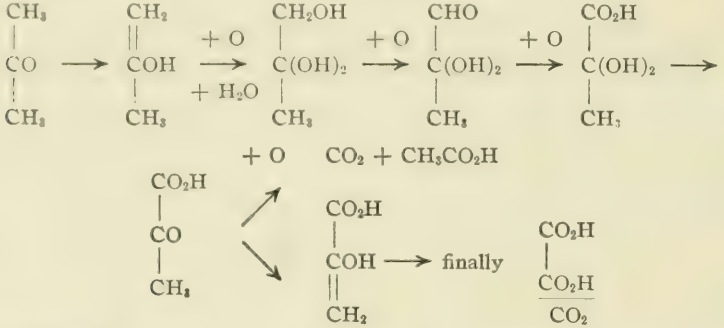
Denis' interpretation of the oxidation of acetone depended on indirect evidence and although apparently not known to Fournier, was confirmed

[1] Pieroni, *Gazz. chim. ital.*, **42**, I, 534 (1912).
[2] *Bull. soc. chim.*, [4] **3**, 256 (1908).
[3] *Monatsh.*, **10**, 782 (1889).

by him so far as the intermediate formation of pyruvic acid is concerned.[1]

The general significance of these results must be that pyruvic acid is a relatively stable intermediate oxidation product of those three-carbon compounds that are capable of giving rise to it. The list of such compounds if all of the rather unstable intermediates like acetol, methyl glyoxal, lactic aldehyde, etc., are included is quite a long one.

On the basis of the above general discussion, it is now possible to construct a scheme for the oxidation of acetone analogous to that of Pieroni and Tonnioli for the formation of iodoform, thus:



This scheme:

(1) readily permits of the explanation of the simultaneous formation of acetic and oxalic acids in the oxidation of acetone;

(2) is in agreement with Denis' conclusions about the properties of acetone in alkaline solutions;

(3) is supported by Fournier's experiments[2] on the formation of pyruvic acid;

(4) is in line with Pieroni and Tonnioli's explanation of the formation of iodoform;

(5) is supported by my own conclusions about the significance of the relative rate of increase in the yield of oxalic acid.

In the above experiments the observations of earlier workers that acetone is not acted upon by potassium permanganate in the absence of alkali was confirmed. The quotation from Denis' paper establishes the fact that it is the isoacetone $CH_2 : COH.CH_3$ in alkaline solutions that is reactive, and that the permanganate in the absence of extra alkali is incapable of producing a sufficient amount by hydrolysis to promote

[1] [It is of importance in this connection to note that Beilstein and Weigand (*Ber.*, 17, 840 (1884)) found pyruvic acid as an intermediate in the oxidation of calcium lactate with permanganate. Moreover the author in some earlier experiments (*Dissertation*, Ohio State Univ., 1912) on the oxidation of propylene glycol with permanganate concluded that pyruvic acid was an intermediate product although unaware at the time of Fournier's experiments on acetone.]

[2] *Loc. cit.*

rapid oxidation. If this interpretation is acceptable the only point in the above scheme that is open to objection, that has not been discussed, is the question as to whether pyruvic acid may enolize in the way indicated. At this point the question may fairly be asked: If the methyl group in acetone can only be attacked after enolization why should that in pyruvic acid be attacked without? Such effects of the ketone group on the adjoining part of the molecule are very general and are discussed by Meyer and Jacobson.[1] Moreover, this effect appears to occur especially strongly in pyruvic acid,[2] as is indicated by the numerous spontaneous condensation reactions to which it is subject,[3] and for which such an enolization constitutes a simple explanation. With enolized pyruvic acid as with other enolized molecules, the velocity of reaction is enormously increased by the presence of alkali. Thus Wolff[4] found that the condensation of pyruvic acid is greatly accelerated by the presence of potassium or barium hydroxide, potassium cyanide or ammonia.[5]

Nothing can be said with certainty about other intermediate stages than the above. In fact it is not certain but what the enolization of the second methyl group takes place before pyruvic acid is reached as for instance with acetol, but more likely with methyl glyoxal. But at whatever stage it does take place or in whatever intermediate condition the compounds may exist (such for instance as the dissociation forms so much used by Nef) it seems clear that the second methyl group is not involved in that portion giving rise to acetic and carbonic acids.

The possible importance of α-keto acids in biology cannot be estimated at present because so little is known about them. Aside from pyruvic acid (which is easily obtained and has been rather much studied) the only

[1] *Lehrbuch der Organischen Chemie*, II, 670 (1907).

[2] [Denis stated (*Loc. cit.*, p. 576) that "enol molecules of pyruvic acid must of necessity be present in the alkaline solution."]

[3] [Meyer and Jacobson, "*Lehrbuch der Organischen Chemie*," III, 1118 (1913); cf. Finck, *Ann.*, **122**, 182 (1862); Böttinger, *Ber.*, **6**, 895 (1873); **8**, 1583 (1875); **9**, 836 (1876); **18**, 609(1885); *Ann.*, **172**, 239 (1874); **188**, 300 (1877); **208**, 122 (1881); Mulder, *Rec. trav. chim.*, **12**, 86 (1893); **13**, 393 (1894); **14**, 297 (1895); de Jong, *Ibid.*, **20**, 81, 365 (1901); **21**, 191, 299 (1902); **25**, 229 (1906); Gault, *Compt. rend.*, **153**, 109 (1911); but especially L. Wolff, *Ann.*, **305**, 125, 154 (1899); **317**, I, 22 (1901).]

[4] *Loc. cit.*

[5] [The earlier experiments on the oxidation of propylene glycol, lactic and pyruvic acids (Evans and Witzemann, *Loc. cit.*) are susceptible to the same interpretation as far as the formation of oxalic acid is concerned. That is pyruvic acid, which as was stated above occurs as an intermediate is enolized by the alkali and it is the oxidation of this enol that determines how much oxalic acid is formed. The experiments of Evans and Day (THIS JOURNAL, **38**, 375 (1916)) on the oxidation of ethyl alcohol with permanganate in the presence of variable amounts of alkali, with the sole production of acetic, oxalic and carbonic acids are susceptible to a similar interpretation on the basis that the intermediate product acetaldehyde is enolized to give vinyl alcohol (McLeod, *Am. Chem. J.*, **37**, 24 (1907).]

SERIES I.

I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.	XIII.	XIV.	XV.	XVI.	XVII.	XVIII.	XIX.	XX.	XXI.
						$KMnO_4$ deficit.														
G. acetone.	G. $KMnO_4$ used.	G. $KMnO_4$ calc.	G. MnO_2 equiv.	MnO_2 equiv. calc.	G. MnO_2 calc.	From $KMnO_4$.	From $KMnO_2$.	G. KOH.	*N* KOH.	G. CO_2.	G. oxalic acid.	G. acetic acid.	% oxalic + CO_2.	% acetic + CO_2.	% acetone unchanged.	% recovered.	G. KOH for K salts.	G. KOH from $KMnO_4$.	Total g. KOH.	G. KOH in MnO_2 ppt.
1... 2	No reduction even on				...	...	.....	0	0	long standing	....	....	....	....	....	...	...	...	...	
2... 2	13.97	14.64	5.41	5.59	7.68	0.67		1.2	0.021	.....	0.436	1.577	14.02	76.21	10.48	100.6	5.49	4.95	4.90	1.25
3... 2	15.53	15.87	6.00	6.21	8.55	0.34		2.43	0.043	1.524	0.642	1.514	20.64	73.17	5.10	98.91	5.78	5 51	6.54	1.40
4... 2	16.86	18.16	6.09	6.74	9.28	1.30		3.63	0.064	.....	0.955	1.477	30.71	71.30	.....	102.0	6 44	5.97	8.08	1.52
5... 2	17.53	17.99	6.84	7.01	9.65	0.46		4.86	0.087	.....	1.020	1.363	32.78	65.84	.....	98.6	6.33	6.21	9.49	1.58
6... 2	17.29	18.42	6.69	6.91	9.51	1.13		6.06	0.108	1.625	1.097	1.336	35.22	64.54	.....	99.76	....	6.12	10.63	1.55
7... 2	17.52	18.72	6.43	7.01	9.64	1.20		7.29	0.130	1.660	1.157	1.298	37.20	62.70	.....	99.9	....	6.21	11.92	1.58
8... 2	16.93	18.46	6.56	6.69	9.21	1.73		8.49	0.151	1.77	1.071	1.363	34.42	65.83	.....	100.2	....	5.94	12.92	1.51
9... 2	16.54	18.26	6.34	6.61	9.10	1.72		9.72	0.173	1.76	1.073	1.325	34.48	64.00	.....	98.5	....	5.86	14.09	1.49
10... 2	16.26	18.49	6.26	6.50	8.95	2.23		10.92	0.195	1.55	1.095	1.358	35.20	65.59	.....	100.8	....	5.73	15.19	1.46
11... 2	18.04	19.48	7.13	7.21	9.93	1.44		12.15	0.216	1.602	1.340	1.166	43.81	56.34	.....	100.1	....	6.39	16.92	1.62
12... 2	18.06	19.62	7.19	7.22	9.94	1.56		14.58	0.260	1.646	1.359	1.204	43.69	58.20	.....	101.9	....	6.40	19.37	1.63
13... 2	16.77	18.83	....	6.70	9.23	2.06		19.44	0.347	.....	1.183	1.305	38.01	63.07	.....	101.1	....	5.95	23.88	1.51
14... 2	17.42	19.50	....	6.97	9.60	2.08		38.88	0.694	1.720	1.485	1.051	47.72	50.78	.....	98.5	....	6.18	43.49	1.57
15... 2	20.09	21.43	7.98	8.03	11.06	1.34		77.76	1.388	2.072	1.979	0.751	63.61	36.28	.....	99.9	....	7.12	83.07	1.81
16... 2	20.06	21.81	8.04	8.02	11.04	1.75		155.52	2.776	.....	2.038	0.726	65.84	35.07	.....	100.9	....	7.10	160.82	1.80

The difference between the values in Cols. IV and V are within the limits of experimental error.

Blank spaces occur where the result was not determined.

SERIES II.

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.	XIII.	XIV.	XV.	XVI.	XVII.	XVIII.	XIX.	XX.	XXI.
							$KMnO_4$ deficit.														
	G. acetone.	G. $KMnO_4$ used.	G. $KMnO_4$ calc.	G. MnO_2 equiv.	MnO_2 equiv. calc.	G. MnO_2 calc.	From $KMnO_4$.	From MnO_2.	G. KOH.	*N* KOH.	G. CO_2.	G. oxalic acid.	G. acetic acid.	% oxalic + CO_2.	% acetic + CO_2.	% acetone unchanged.	% recovered.	G. KOH for K_2 salts.	G. KOH from $KMnO_4$.	Total g. KOH	G. KOH in MnO_2 ppt.
1	2	No reduction even on long standing				...	...	The differences between the values Cols. IV and V are within the limits of experimental error.	0	0					....	....	....	...	...	....	...
2	2	14.25	14.47	5.34	5.70	7.83	0.23		1.2	0.021	1.67	0.368	1.497	11.82	72.34	12.1	96.26	5.09	5.05	4.97	1.28
3	2	12.61	13.55	4.94	5.04	6.93	0.94		2.43	0.043	1.31	0.492	1.36	15.82	65.70	22.2	102.7	5.02	4.47	5.77	1.13
4	2	16.85	17.12	6.63	6.74	9.26	0.27		3.63	0.064	1.70	0.800	1.51	25.72	73.04	0.60	99.36	6.21	5.97	8.08	1.52
5	2	17.29	17.49	6.79	6.91	9.51	0.20		4.86	0.087	1.68	0.947	1.39	30.48	67.15	0.53	97.6	6.23	6.12	9.42	1.55
6	2	17.22	17.51	6.93	6.9	9.47	0.29		6.06	0.108	1.67	0.978	1.36	31.46	65.65	0.21	97.1	....	6.10	10.61	1.55
7	2	17.07	17.41	6.85	6.83	9.39	0.34		7.29	0.130	1.75	0.966	1.36	31.06	65.65	0.60	96.7	....	6.05	11.81	1.53
8	2	16.89	17.77	6.68	6.75	9.29	0.88		8.49	0.151	1.70	1.019	1.35	32.78	65.12	0.46	97.9	....	5.98	12.95	1.52
9	2	17.98	18.96	7.18	7.19	9.78	0.98		9.72	0.173	1.73	1.204	1.31	38.45	63.28	0.46	101.7	....	6.30	14.40	1.62
10	2	16.93	18.15	6.97	6.77	9.31	1.22		10.92	0.195	1.71	1.096	1.31	35.26	63.40	0.27	98.6	....	6.00	15.40	1.52
11	2	16.85	18.02	6.83	6.74	9.26	1.17		12.15	0.216	1.65	1.082	1.31	34.75	63.40	0.50	98.15	....	5.97	16.60	1.52
12	2	16.98	18.38	7.03	6.79	9.33	1.40		14.58	0.260	1.56	1.150	1.28	36.97	61.94	0.45	98.9	....	6.02	19.07	1.53
13	2	17.13	18.56	6.69	6.85	9.42	1.43		19.44	0.347	1.67	1.290	1.14	41.46	55.26	0.66	96.7	....	6.07	23.99	1.42
14	2	17.88	19.63	6.85	7.15	9.83	1.75		38.88	0.694	1.65	1.591	0.946	51.15	45.69	.....	96.8	...	6.34	43.61	1.61
15	2	20.12	21.72	7.90	8.04	11.06	1.60		77.76	1.388	1.82	1.996	0.774	64.18	37.39	.....	101.5	....	7.13	83.08	1.81
16	2	20.51	21.72	8.19	8.20	11.28	1.21		155.52	2.776	1.86	2.027	0.726	65.49	35.06	.....	100.5	....	7.26	160.9	1.85

one well known is glyoxylic acid. C. Neuberg's discussion of the sugar exchange of the cell[1] indicates the possible great importance of pyruvic acid in sugar metabolism in plants and animals. The possible importance of the homologs of pyruvic acid in fat metabolism has apparently never received serious consideration, although a moment's thought will convince one that they are capable of tremendous importance in this connection. The author hopes to deal with this suggestion in greater detail elsewhere.

Résumé.

Experiments were made on the oxidation of acetone with potassium permanganate in the presence of variable amounts of alkali in order to see whether the oxidation of this substance, which is not perceptibly oxidized by air even in the presence of alkali, nor by permanganate except in the presence of alkali, is also accompanied by spontaneous oxidation resulting in the utilization of less than the calculated amount of permanganate. It was found that the deficit in the permanganate consumed that was observed in the oxidation of glucose[2] also occurs here. In the earlier paper it was concluded that this deficit can be explained in one of three ways: (1) a large part of the manganese dioxide is reduced to a manganous compound and subsequently oxidized to manganese dioxide by the air; (2) the alkali serves as a catalyst in facilitating the oxidation of the organic compound; (3) the combined effect of (1) and (2). From the above experiments we may conclude that (1) is true unless the atmospheric oxygen acts upon some lower stage in the oxidation of acetone.

In the absence of alkali acetone is not perceptibly oxidized by permanganate at room temperature. In the presence of small amounts of alkali the oxidation is incomplete—unchanged acetone remains at the end of the oxidation. In the presence of sufficient alkali the acetone is oxidized solely to acetic, oxalic and carbonic acids in such a way that one molecule of carbonic acid is formed for each molecule of acetic and oxalic acids. The proportion of oxalic acid increases gradually but continually with increasing alkalinity. This is different from what was observed for glucose where the maximum yield of oxalic acid was obtained as soon as the available alkali exceeds that required for the formation of normal salts of the acid products, and is interpreted as signifying that the formation of oxalic acid depends on a molecular transformation that is catalyzed or dependent on alkali. Such a transformation would be the conversion of pyruvic acid into its enol isomer, $CH_2 : COH.COOH$. It was shown by the previous results of other workers on the oxidation and chemical properties of acetone and by the general characteristics of ketone deriva-

[1] C. Oppenheimer, *Hdb. d. Biochem. d. Menschen u. Tiere, Erganzungsb.*, **1913**, pp. 569–611.

[2] *Loc. cit.*

tives that such a transformation would tend to occur. On the basis of all the evidence presented there cannot be any doubt that the enolization of pyruvic acid represents a logical and simple explanation of the deviation of the oxidation products from mostly acetic and carbonic acids to mostly oxalic and carbonic acids.

From all the above discussion it is clear that alkali performs at least the following functions in the oxidation of acetone:

(1) It gives rise to the reactive enol—isoacetone;

(2) It brings about the enolization of pyruvic acid;

(3) It neutralizes the acids formed and thus interferes with their further oxidation;

(4) It alone determines the velocity of oxidation;

(5) It catalyzes the absorption of atmospheric oxygen.

The point of view developed in this paper concerning the mechanism of the oxidation of acetone is of great interest in the interpretation of the oxidative breakdown of fatty acids. This relation will be discussed in a subsequent paper.

CHICAGO, ILL.

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THE INFLUENCE OF OXYGEN UPON INFLAMMATORY REACTIONS

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Experiments by Amberg and Knox (1), Amberg (2), and Arkin (3) have shown that various inflammatory reactions are markedly inhibited by intravenous injections of the sodium salts of o-iodoso- and o-iodoxy-benzoic acids. In these experiments the agents producing the inflammation had been administered intracutaneously. Bruce (4), Chiari and Januschke (5) reported an inhibiting effect of various substances on the inflammatory edema of the conjunctiva of the rabbit produced by the instillation of mustard oil. They took the precaution of lifting the lower lid well away from the eye ball, dropping the oil in the sac thus formed. In this manner it is possible to obtain rather uniform results. In the following series of experiments our substances were injected intravenously while the mustard oil was instilled in the conjunctival sac. The instillation of mustard oil in the eye of the normal rabbit is followed immediately by a spasm of the lids and weeping. Within a short time the spasm relaxes, the eye opens and the conjuctiva is seen deeply congested with the pupil contracted. Edema of the palpebral and ocular conjunctiva develops, reaching its maximum in about an hour. The lids stand out well and there is some eversion of edematous tissue. The cornea can no longer be exposed entirely. Keeping step with the development of the edema the conjuctiva blanches. The next day the edema has usually diminished very much, while the hyperemia has reestablished itself. The lids may be found glued together by a

creamy exudate filling the conjunctival sac. The cornea is opaque. The effect of the intravenous injections of the drug mentioned will be illustrated by a few examples.[1]

Rabbit 7. March 4, 1914. 1300 grams

2.45 p.m. 10 cc. $\frac{N}{20}$ sodium iodoxybenzoate.
2.50 p.m. 1 drop mustard oil in left conjunctival sac. Spasm of lids.
2.55 p.m. Eyes open. Hyperemia of conjunctiva.
3.00 p.m. Same.
3.30 p.m. Eye appears normal.
4.00 p.m. Same.
7.45 p.m. Same.

March 5, 1914, 10 a.m. A little purulent exudate. Otherwise nothing to be seen.

Rabbit 9. March 5, 1914. 1170 grams

3.32 p.m. 10 cc. $\frac{N}{20}$ sodium iodoxybenzoate.
3.34 p.m. 1 drop of mustard oil in left conjunctival sac. Spasm of lids.
3.50 p.m. Eye open, no edema, slight redness.
4.55 p.m. Same.

March 6, 1914, 10 a.m. Lids glued together, creamy pus in sac. Corneal opacity.

Rabbit 99. June 7, 1914. 920 grams

3.20 p.m. 5 cc. $\frac{N}{20}$ sodium iodoxybenzoate immediately followed by 1 drop of mustard oil in right conjunctival sac.
3.50 p.m. Slight redness, weeping, no edema.
4.20 p.m. Redness, slight edema.
5.20 p.m. Marked edema.

June 8, 1914, 10 a.m. Slight opacity of cornea, edema, redness.

Rabbit 19. March 10, 1914. 2075 grams

3.00 p.m. 10 cc. $\frac{N}{20}$ sodium iodosobenzoate, then mustard oil in right conjunctival sac.
3.05 p.m. Eye open, weeping, hyperemia.
3.15 p.m. Eye closed, weeping, redness, no edema.
3.30 p.m. Eye open, no edema, slight hyperemia.

[1] In a part of the work we were assisted by Dr. P. S. Chancellor.

4.00 p.m. Same.
4.30 p.m. Eye apparently normal.
5.30 p.m. Same.
7.35 p.m. Slightly pink, otherwise normal.
March 11, 1914, a.m. Eye apparently normal.

It may be remarked that all our experiments were accompanied by controls. The intravenous injection of sodium iod-benzoate had no inhibiting effect, and the protocol of one of these experiments may be taken as an illustration of the course of the conjunctival mustard oil reaction in an untreated animal.

Rabbit 28. March 13, 1914. 975 grams

3.40 p.m. 10 cc. $\frac{N}{20}$ sodium iod-benzoate; immediately afterwards 1 drop of mustard oil in left conjunctival sac.
3.45 p.m. Eye closed, weeping, some redness.
4.00 p.m. Considerable edema, slight redness and weeping.
4.10 p.m. Marked edema.
4.20 p.m. Edema progressed.
4.30 p.m. Very pronounced edema. Difficult to expose more than about half of the cornea.
5.00 p.m. Edema about same.
9.25 p.m. Edema less, white secretion, lids glued together.
March 14, 1914. Lids glued together, creamy exudate, redness, edema much diminished.

Some control experiments were also made with 5 per cent NaCl, of which 10 cc. were injected intravenously in two rabbits of 1410 and 1210 grams weight without any effect on the intensity of the reaction. Furthermore, control experiments with sodium salicylate seemed advisable, for Jahn (7) detected traces of salicylic acid in the urine of rabbits after intravenous injections of sodium iodoso benzoate. Januschke (6) gave rabbits of 1400 to 1600 grams, in half hourly intervals, three doses of 0.5 gram sodium salicylate subcutaneously. At the time of the last injection mustard oil was instilled in the eye. A very marked inhibition of the inflammation was noted. We injected three rabbits of 1330 to 1520 grams each with 10 cc. $\frac{N}{10}$ sodium salicylate intravenously, without any definite inhibiting effect on the conjunctival mustard oil reaction.

When the edema is completely or nearly completely inhibited by sodium iodoxybenzoate, the weeping and the hyperemia occuring immediately after the instillation of the mustard oil may disappear within about half an hour. Sometimes, it is hardly possible to determine which eye has been used. Rather frequently the eyes of control animals with a rapidly progressing edema, show less congestion than the eye of animals whose edema is markedly inhibited. However, in spite of a nearly complete inhibition of the edema sometimes the cornea was found cloudy the next day and there was considerable creamy exudate. In some cases as in many of the controls the opacity of the cornea was very pronounced, and persisted, while in others the eye recovered entirely. The cloudiness of the cornea may begin three to four hours after the instillation of the oil.

We may summarize our results by saying that the intravenous injection of sodium iodoso- or iodoxy-benzoate inhibits the inflammatory edema produced by mustard oil in the conjuctiva of the rabbit, while the sodium iod-benzoate has no such effect. It appeared that the degree of inhibition is roughly proportional to the dosage. Furthermore, the sodium iodosobenzoate seemed to be more effective than the sodium iodoxybenzoate.

Some animals having received intravenous injections of sodium iodoso- and iodoxy-benzoate, with consequent marked inhibition of their conjunctival mustard oil reactions, were sacrificed one hour after the injection. The viscera did not show any macroscopical or microscopical lesions.

It had been noted that the administration of our substances was followed by some diarrhoea. Sometimes this diarrhoea became very profuse. When this happened the experiments were excluded from consideration. Nevertheless, it became desirable to determine definitely whether any importance attached to the diarrhoea as a factor in the observed inhibition of the mustard oil reaction. It should be pointed out that quite pronounced diarrhoea had been seen occasionally in control animals which gave very good inflammatory reactions. Two possibilities had to be considered; the first was the actual loss of

fluid from the organism, the second. a possible effusion of fluid in the small intestines without a manifest diarrhoea. Some few attempts at obtaining a pronounced but controlable diarrhoea in rabbits by means of a combined treatment with saline catharctics and pilocarpin or physostigmin, did not yield reliable results. Our question was answered satisfactorily, however, in the following manner: A female rabbit, after expressing the contents of the bladder, weighed 1800 grams just before the experiment was started. It received a continuous injection of a 0.4 per cent solution of iodoxybenzoic acid as sodium salt in the marginal ear vein by means of a Woodyatt (8) motor syringe. Twenty-three minutes after the beginning of the injection, when the rabbit had received 12 cc. of the solution, 2 drops of mustard oil were instilled in the conjunctival sac, without interrupting the injection, which was continued for forty-seven minutes longer. During this time 26 cc. were injected, giving a total of 38 cc. At the end of the injection the rabbit passed spontaneously a small well formed stool. A small amount of urine and some more fecal balls were expressed. The rabbit weighed 1830 grams. From then on until it was killed two and one-half hours after the instillation of the mustard oil no more stool or urine was passed. In the beginning of the experiment there was some secretion from eyes and mouth, the latter keeping up throughout the injection, but as can be seen from the weight of the animal this salivation was very moderate indeed. The inhibition of the mustard oil reaction, while not complete, was very marked. At the same time the mustard oil was applied to this animal a control rabbit was treated alike and responded in the usual manner with a fully developed edema, in spite of the fact that thirty minutes after the instillation of the mustard oil it passed some rather fluid stools and continued to do so for about 20 minutes. It was also killed two and one-half hours after the application of the mustard oil.

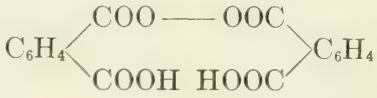
The rectum of the injected animal contained well formed dry fecal matter. The content of the large intestine seemed to be of about the usual consistency and the small intestines did not appear unusually filled. The rectum of the control animals con-

tained semisolid unformed fecal matter and the contents of the large intestine were decidedly more moist. The experiment was repeated with the same result as far as the effect of the intravenous injection of the sodium iodoxy benzoate was concerned the animal being killed seventy-five minutes after the instillation of the mustard oil. The fortunate accident of a control with a marked diarrhoea did not reoccur. It may be mentioned that several times rabbits having received several drops of mustard oil had a transitory slight diarrhoea, that is, they passed rather more fecal balls, decidedly moister and less well formed than usual.

We have here, then, a very marked inhibition of the mustard oil reaction without a trace of a diarrhoea while a control with a rather profuse diarrhoea did not show any inhibition. Neither did we have any indication of an effusion of fluid anywhere in the organism, a conceivable factor in the inhibiting action. Furthermore, neither in these experiments nor in those mentioned above where a post mortem examination was made, did we have any indication of any irritation caused by our injected substances, a point to which we will have to refer further on.

It now became our aim to study the effect of other organic substances containing chemically active oxygen in other combinations, and furthermore some inorganic salts with active oxygen. The selection of substances suitable for our purposes is very limited (see Grove and Loevenhart (9)).

In previous experiments (2) with the disodium salt of diphthalic peroxide (see Bayer and Villiger (10)).

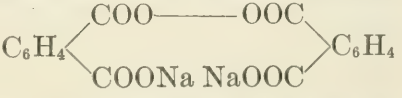


no inhibiting effect could be seen. The solution then used was $\frac{N}{20}$ but contained only 53 per cent of the theoretical amount of active oxygen. The solution now used was $\frac{N}{10}$ and contained 95.6 per cent of the theoretically required active oxygen. The solution prepared with the theoretical amount of NaOH was still very slightly acid. The intravenous injections were made

immediately after the preparation of the solution and the utmost care had to be observed in making the injections. The mustard oil was given intracutaneously, 0.1 cc. of a 1 per cent solution in olive oil. A rabbit of 2200 grams and another of 2060 grams received each 10 cc. and a third rabbit of 1380 grams received 6.5 cc. of the $\frac{N}{10}$ sodium perphthalate. The inflammatory reactions were measured in mm. as in previously reported experiments (2) and seven and one-half hours after the injections,[2] with the following average results:

	THREE HOURS	SEVEN AND ONE-HALF HOURS
	mm.	*mm.*
Controls	32	41
Experiment	25	35

After three hours the cutaneous swelling of the treated animals was rather diffuse, ill defined and not pronounced and we had at this time an inhibition more marked indeed than indicated by the measurements. This inhibition became less and less pronounced as the time advanced. In another series we had only one successful experiment, several other animals dying within a few minutes after the intravenous injections. In this, a rabbit of 1080 grams received 6 cc. of a $\frac{N}{10}$ solution, and mustard oil in the eye; this animal had a marked inhibition of the inflammatory process without showing any toxic symptoms. The dry preparation from which the solution was made contained 5.35 per cent active oxygen, instead of 4.85 per cent, as required by theory. The solution required decidedly more than the theoretical amount of NaOH. The final solution contained about 90 per cent of the theoretical amount of active oxygen and may have been a mixture of



[2] The figures given for the measurements of the intracutaneous reactions were obtained by measuring two diameters in millimeters and taking the mean. Then the average of the reactions in a series of animals was struck. The figures therefore represent the average diameter of the reactions in millimeters.

and $C_6H_4\begin{matrix}COOONa\\COONa\end{matrix}$ and sodium phthalate. Control experiments with $\frac{N}{10}$ sodium phthalate were negative. The sodium salt of diphthalic peroxide exercises undoubtedly an inhibiting influence on the mustard oil inflammation.

Solutions of sodium iodate and sodium periodate were prepared by neutralizing the corresponding acids with NaOH. The concentrations used for intravenous injection was $\frac{N}{20}$. In one set of experiments, 0.1 cc. of a 2 per cent mustard oil solution was injected intracutaneously. Four animals from 2100 to 2340 grams received each 6.6 cc. sodium iodate, four animals from 2000 to 2400 grams 5 cc. sodium periodate four animals received 100 cc. O_2 gas intraperitoneally and four served as controls. The average results were as follows.

	TWO HOURS	FOUR HOURS	SIX HOURS
	mm.	*mm.*	*mm.*
Controls	30	39	46
Sodium iodate	24	33	41
Sodium periodate	28	31	38
Oxygen gas	30	39	44

In these experiments the iodates exercised undoubtedly an inhibiting effect, showing itself, as in the experiments with diphthalic peroxide, less in the area of the swelling than in its height. The average size of the reactions of the animals treated with sodium periodate two hours after the injection exceeded those of the animals treated with sodium iodate. Nevertheless the reactions of the periodate animals were decidedly the least intense ones throughout. The fact that the differences in the intensity of the reactions is not shown as well in their area as in those experiments reported in an earlier paper (2) may be due to the greater dilution of the mustard oil in these experiments.

Sets of two animals each served for experiments with instillation of the mustard oil in the eye and intravenous injections of $\frac{N}{10}$ solutions of sodium iodate and sodium periodate, and $\frac{N}{10}$

sodium iodide. Two animals of 1304 grams received each 10 cc. sodium iodate solution, two of 1140 and 1300 grams received each 10 cc. sodium periodate solution, two of 1120 grams each 10 cc. NaI while two served as controls. There was no difference between the reactions of the controls and those of the sodium iodide animals, but a marked inhibition was caused by the iodates with a decidedly stronger effect of the periodate.

It is to be noted that injections of 2 to 5 cc. $\frac{N}{20}$ sodium periodate in the femoral vein of etherized cats of 2 kgm. weight did not have any very marked effect on respiration or blood pressure. On injection of a fatal dose the respiration becomes gradually slower and shallower until it ceases. There is no effect on the respiration comparable to that of sodium iodoso and iodoxy benzoate (Grove and Loevenhart (9)).

Trials with sodium perborate were given up. The injection is mechanically very difficult and the animals die very readily. Of a series of experiments only one animal of 625 grams survived 10 cc. of a 0.89 per cent solution. There was some doubtful inhibition of the eye reaction.

Ammonium persulfate, of which a $\frac{N}{20}$ neutralized solution in 10 cc. doses was injected in three rabbits of about 1000 grams, did not produce a noteworthy, if any, inhibition of the conjunctival mustard oil reaction. A peculiar result was obtained with a commercial solution of sodium hypochlorite. The alkalinity of this solution was equal to $\frac{N}{10}$ and 1 cc. required 10.58 cc. $\frac{N}{10}$ thiosulfate solution. One animal of 1590 and another of 2290 grams received each 10 cc. of a 1:5 freshly prepared solution intravenously. A third animal of 1400 grams received 10 cc. of a 1:10 solution. In all three animals mustard oil was instilled in the eye. There was perhaps some lag in the development of the edema. The hyperemia was very intense and there was a bloody exudation. The first two animals survived and the next day showed less traces of their hemorrhages than was to be expected, indeed it is questionable if anything unusual would have been noted if it had not been for the peculiar character of the reactions the day before. From both of these animals some bloody urine was obtained. The third animal whose hemor-

rhagic edema had become pronounced one and one-half hours after the application of the mustard oil, was found dead in its cage the next morning. The eye lids still presented a marked edema suffused with blood. A part of the lids and also the eye ball were destroyed and bloody fluid filled the socket. Coagulated blood was in the cage and the urine receptacle contained very bloody fluid. The animal seemed to have bled to death from a ruptured vessel of the eye. The point we wish to emphasize in this connection is this: that the character of the inflammation may be altered by the introduction of certain substances into the circulation. In this case it is a substance having evidently an injurious effect on the vessels, as may be inferred from the appearance of bloody urine. In addition it should be noted that animals dying from the effect of the hypochlorite solution present hemorrhages as the most striking feature.

For several reasons we tried to administer substances by the intraperitoneal route, the effect of which we wished to study on the mustard oil inflammation. It was thought particularly desirable to study the effect of benzoyl peroxide, which had proved of value clinically as pointed out by Loevenhart (11). Benzoyl peroxide is very slightly soluble in non-irritating solvents. The intravenous injections of sodium iodoso- and iodoxy-benzoates are time consuming and not always very easy, and these compounds can not be given subcutaneously, because they produce severe irritation. Iodoxy-benzoic acid was given in solution as the sodium salt; iodoso-benzoic acid was given in suspension in saline, its solution as sodium salt having proved very irritating in the experiments of Grove and Loevenhart. Benzoyl-peroxide was also given in suspension through a small incision in the abdominal wall. The dosage of the iodoxy- and the iodoso-benzoic acid was the same as in the previous experiments, and the inhibiting effect was also apparently about proportional to the dose. The mustard oil was instilled in the conjunctival sac. As usual, sodium iod-benzoate had no inhibiting effect. Benzoyl-peroxide was given in large doses, 0.75 to 1 gram each, to two animals of 1500 to 2000 grams. One animal of each set showed a very pronounced inhibition, the others showed

about the same grade of edema as the controls which received 7.5 and 10 cc. of a 10 per cent solution of sodium benzoate intraperitoneally. Two animals of about 1600 grams received each 10 cc. of a 10 per cent solution of sodium chloride intraperitoneally without any effect on the reaction.

In order to test the possible effect of the introduction of foreign substances in the peritoneal cavity on the mustard oil reaction of the eye, a number of animals received Kaolin (Merck) in 2.5 and 10 per cent suspensions. It was found that as little as 0.1 gram Kaolin given to an animal of about 900 grams may produce a marked inhibition, but not always. As the result of fourteen experiments on animals weighing 800 to 1900 grams, killed one and one-half to twelve hours after the injection, we may state that the degree of inhibition is roughly parallel to the intensity of the peritoneal lesions produced by the injections. A marked inhibition was also noted on intraperitoneal injections of 10 cc. of a 5 per cent suspension of animal charcoal (Blutkohle, Kahlbaum) in three animals. It may be mentioned that the intracutaneous injection of small amounts of Kaolin in man is very painful and leads to a strong inflammatory reaction.

One of two animals received 1 cc. of a $\frac{N}{20}$ solution of sodium iodoxybenzoate per 100 grams bodyweight intraperitoneally, another a like dose of iodoso-benzoic acid in suspension. Both showed good inhibition of their mustard oil reaction, which in the latter case was nearly complete up to the time the animals were killed, that is, one hour after the treatment. The first animal showed only doubtful signs of peritoneal irritation, the other marked signs such as great excess of free fluid and numerous haemorrhages in the omentum. Three rabbits received sodium iodoxybenzoate, three iodoso-benzoic acid in suspension. All animals showed marked inhibitions. They were killed after three hours, when those treated with iodoso-benzoic acid had bloody fluid in the peritoneal cavity and fine haemorrhages on the serosa of the abdominal wall as well as of the large and small intestines. The animals treated with sodium iodoxybenzoate solution had no excess fluid and the pin-point haemorrhages were much less numerous. One of the animals having received

10 cc. 10 per cent NaCl was sacrificed after thirty-five minutes, when the edema of the lids was already very marked. There was a considerable excess of perfectly clear fluid in the peritoneal cavity while the serosa of the abdominal wall was somewhat injected.

The results of experiments where substances are administered intraperitoneally must be interpreted with great caution. While it is very doubtful whether in the case of sodium iodoxybenzoate, peritoneal irritation enters into consideration with regard to its inhibiting action, we will only very briefly refer to a series of experiments undertaken to see whether the sodium iodoxy-benzoate can exercise any influence when the edema is once under way. The administration ten minutes after the application of the mustard oil, when a slight degree of edema exists, causes a pronounced inhibition. When given an hour after the instillation of the mustard oil, that is at a time when the edema is rather fully developed, no effect whatever was noted in some cases, in others there may have been a slightly beneficial effect, but in no case was this very striking.

In order to obtain some further information concerning the influence of irritation elsewhere on the cause of the mustard oil reaction of the eye, a few experiments were made in which rabbits received mustard oil intraperitoneally or subcutaneously. When 0.1 cc. of a 1:20 dilution of mustard oil was injected intraperitoneally in two rabbits of about 1200 grams no definite inhibition of the conjunctival inflammation was noted, with 0.3 cc. one animal of 1350 grams did not show any definite inhibition, the other of 1250 grams did. Four hours after the injection the animals were killed. Those without any definite inhibition had hardly any excess fluid in the peritoneal cavity and only a few pin point haemorrhages on the serosa of the large intestine, the one with marked inhibition had more fluid, a small area of intense injection of the peritoneum at the site of the injection and in several places the peritoneal covering of the large intestine was studded with small haemorrhages. Another animal of 1350 grams had received 0.5 cc. No definite edema developed before the lapse of one and one-half hours, then the edema be-

came well marked four hours after the injection when the animal was killed. The animal had been ill; it was restless, had a very rapid respiration, and was somewhat cyanotic. These symptoms subsided but gradually. At the time it was killed it appeared quite normal. The peritoneal cavity was filled with clear fluid containing some flakes. A gelatinous coagulum was lifted out of the fluid. The serosa of the abdominal wall as well as that of the small intestine was congested, but nowhere intensely. The serosa of the large intestine was the seat of very fine haemorrhages which were rather numerous in places. We have here a development of quite a fair degree of conjunctival edema in spite of the very marked effusion elsewhere. Two animals of 1275 and 1475 grams received each 0.5 cc. 1:10 mustard oil under the skin of the back. Up to the time the animals were killed, that is for three hours, the development of the conjunctival edema of the first animal was very markedly inhibited, that of the second animal not much. The lesions produced by the subcutaneous injections did not differ appreciably in intensity.

The deduction we can draw from these experiments is that a certain degree of inhibition of the mustard oil inflammation of the conjunctiva may be brought about by irritation in other parts of the body, be it in the peritoneal cavity or elsewhere, as under the skin. These experiments call to mind the result of R. Winternitz (12) who studied the inhibiting action of etheral oils on inflammation. He describes experiments with the instillation of a weak solution of bee poison in the conjunctival sac of the rabbit. An animal treated per os with santal oil did not show any reaction while the untreated one showed some conjunctival hyperemia. Furthermore, if we are not mistaken, these experiments are the first to give some support to the clinical conception of the value of counter irritation in inflammation.

In the following experiments we tried to study the effect of diminished tissue oxidations on the mustard oil inflammation.

In a series of experiments rabbits were exposed to a reduced partial pressure of oxygen keeping the atmospheric pressure unchanged. The apparatus of Kolls and Loevenhart (13) was

used. The mustard oil was given intracutaneously, 0.1 cc. of a 1 per cent solution in olive oil, prepared freshly for each series. Four rabbits with four controls served for the first experiment. The four rabbits were put in the apparatus and the oxygen content gradually reduced. From the time the oxygen had dropped to 12.4 per cent until the mustard oil was injected, sixteen hours elapsed. During this time the oxygen tension, with exception of the first reading, varied from 10.7 to 9.1 per cent. The CO_2 content did not rise above 0.15 per cent. The removal of the animals from the apparatus was rather difficult taking one and one-half hours, whereby the oxygen rose to 13.8 per cent. After the injection the animals were returned to the apparatus together with two larger rabbits, in order to reduce the oxygen tension more rapidly. In two hours, when the oxygen had dropped to 12.3 per cent the reactions were measured. On the return to the apparatus the oxygen had risen to 14.4 per cent. In the next four and one-half hours the oxygen dropped to 8 per cent. The animals were taken out, the reactions were measured and the animals returned to 13.1 per cent oxygen which fell in the course of the next ten hours to 6.4 per cent, while the CO_2 at the end of this time was 0.24 per cent. The weights of the control animals were (1) 600 grams, (2) 600, (3) 630, (4) 2280. That of the others (1) 760 grams, (2) 640, (3) 680, (4) 1960. The measurements of the reactions in mm. were as follows:

		1	2	3	4
		mm.	*mm.*	*mm.*	*mm.*
After two hours	Controls	Indefinite	29	27	24
	Experiment	42	27	20	33
After seven and one-half hours	Controls	32	30	45	36
	Experiment	62	35	29	44

The reaction of control 2 was less intense than that of its mate although after two hours the size of the two reactions was about equal. After about twenty hours the reaction of the controls

1, 2 and 3, had diminished very much in intensity while that of 4 had increased. The reaction of 3 had nearly disappeared in the apparatus, 1 and 4 did not show any marked change and in 2 there was an increase. The conclusion from this series of experiments is that on the whole the reactions of the animals exposed to low oxygen tension were somewhat more intense than those of the control.[3]

Two sets of four animals each were used in the second experiment. Here four animals first received their mustard oil injection and were then put in the apparatus at 9.7 per cent oxygen. This time they were taken out after two hours without any difficulty and after measuring the reactions returned to 11 per cent oxygen. They remained in the apparatus three hours. During this time the oxygen dropped to 8.9 per cent rising again at the end to 9.7 per cent. The CO_2 during the first two hours was 0.3 per cent and rose to 0.67 per cent. The weight of the control animals was (1) 2540, (2) 2000, (3) 1660, (4) 1460, that of the others (1) 2450, (2) 1880, (3) 1640, (4) 1120.

		1	2	3	4	REMARKS
		mm.	*mm.*	*mm.*	*mm.*	
Two hours	Control	28	34	34	32	Control (1) showed very little swelling
	Experiment	29	39	40	34	
Five hours	Control	38	41	41	38	
	Experiment	33	39	53	44	

All the reactions showed about the same degree of elevation, firmness and reddening, so that there was no noteworthy difference in the intensity of the reactions.

[3] At the end of the experiment the animals were rather quiet in the apparatus and the respiration was rapid and jerky. After the animals had been taken out the oxygen tension was 7.1 per cent the CO_2 0.24 per cent. This atmosphere did not support a flame; a candle, an alcohol flame, a benzene flame and a burning match were extinguished. A rabbit (control) of 600 grams and another one of 2280 grams were put in and left there for ten minutes. The animals were quiet and their respiration was somewhat labored. After removal of the animals the atmosphere of the box did not support combustion of the flame.

Again two sets, each of four animals, served for the next experiment. Here the animals were introduced into an atmosphere containing 9.8 per cent O_2. For the greater part of the time the oxygen varied between 7.2 and 8.1 per cent. The CO_2 did not rise above 0.18 per cent. After a sojourn of twenty hours in that atmosphere the mustard oil was injected and the rabbit returned to 10 per cent O_2, the whole procedure of removing the rabbits and measuring the swelling taking not more than 15 minutes. Three hours later with the oxygen at 9.1 per cent the animals were taken out easily the reactions measured and the animals returned for three and one-half hours. The oxygen dropped from 9.8 to 9 per cent, and the CO_2 rose to 0.37 per cent. The controls weighed (1) 2280, (2) 2360, (3) 2380 (4) 2340, the others (1) 2000, (2) 2000, (3) 1940, (4) 1820.

		1	2	3	4
		mm.	*mm.*	*mm.*	*mm.*
Three hours	Controls	33	36	28	34
	Experiment	31	22	20	27
Seven and one-half hours	Controls	35	50	37	40
	Experiment	35	31	32	40

The exposure to an atmosphere of low oxygen tension had certainly not resulted in more intense reactions, to the contrary, the reactions of the control animals were somewhat more intense. One of the animals (no. 2) had shown marked symptoms about five hours after being put in the apparatus, it had a marked dyspnoea and was staggering. The oxygen was at about 7.5 per cent, the CO_2 at 0.18 per cent. On admitting oxygen (to 8.1 per cent) the animal improved. The next morning with 7.2 per cent O_2 and 0.1 per cent CO_2 the same animal showed lachrymation and marked dyspnoea, while the other animals moved but little and were perhaps slightly dyspnoeic. At the end of the experiment all the animals were well and lively. The exposure to atmospheres with such low oxygen tension does not seem to be tolerated equally by all animals.

In the last series five animals were kept in the apparatus eighty-four hours before being injected with mustard oil. During this time the highest oxygen content was 12.9 per cent, the lowest 9.8 per cent, most of the time it varied but little from 11 per cent. The CO_2 content rose once as high as 2.04 per cent apparently without causing any discomfort to the animals. After the injection the animals were kept in the apparatus five hours before the reactions were measured, the oxygen dropping gradually from 12.6 to 11.25. The taking out required more time (thirty-eight minutes) and the oxygen rose to 12.7 per cent falling during the next five hours to 11 per cent. After measuring the reactions the animals were kept about thirteen hours longer at about 11 per cent O_2. The CO_2 did not rise above 0.53 per cent. The controls weighed (1) 1480, (2) 820, (3) 1500, (4) 1460, (5) 1780. The other animals were weighed before entering the apparatus and at the end of the experiment (1) 1260, 1200, (2) 1100, 1060, (3) 1540, 1360, (4) 1400, 1250, (5) 1640, 1580.

		1	2	3	4	5
		mm.	*mm.*	*mm.*	*mm.*	*mm.*
Five hours	Controls	32	34	34	34	37
	Experiment	39	44	32	38	35
Ten and one-half hours	Controls	39	45	38	49	41
	Experiment	50	62	39	47	41

At the end of the experiment most of the reactions had diminished very much and similarly in both sets.[4] This experiment does not indicate a definite influence of the prolonged previous exposure to a relatively low oxygen tension of the atmosphere on the mustard oil inflammation, one way or another.

Two other methods were tried that promised some interference with oxidations in the organism. Since these experiments were rather of a tentative nature we will refer to them but

[4] During the experiment the temperature in the apparatus did not vary more than 2.2°C.

briefly. About a third of the calculated amount of blood was withdrawn from two rabbits before the institution of mustard oil in the eye. In both cases the edema required one and one-half to two hours to reach a marked degree and it did not develop as fully as in the controls. There was a considerable inhibition.

Four animals were exposed to illuminating gas. The effects of intoxication with American illuminating gas are according to Yandell Henderson, (14), practically those of CO. These experiments were done very roughly. In three animals with more or less severe symptoms of intoxication there was a corresponding inhibition. One animal that received the mustard oil showed only a little unsteadiness when taken out of the box. This animal quickly developed a very pronounced reaction but since undiluted mustard oil was instilled in the eye it is very difficult to say whether there was a definite increase, even though the edema had hardly diminished the next day.

A number of animals received intravenous injections of sodium cyanide preceding the instillation of mustard oil in the conjunctival sac. Since it is somewhat difficult to distinguish definitely an increase of the reaction when undiluted mustard oil is used, these experiments were made with a drop of mustard oil diluted with an equal volume of olive oil. With a dilution 1:1 there occurred always a rather marked edema about an hour after the instillation and this dilution had the further advantage that after a lapse of about twenty hours the lesions are as a rule not pronounced, so that the result of a more intense reaction can still be seen at that time. The sodium cyanide solution was always freshly prepared from a titrated solution of HCN and contained 0.62 mgm. HCN as sodium salt in a volume of 10 cc. the solution being made with saline.

Rabbit 153. 1590 grams. Received 5.5 cc. NaCN solution, it showed some embarrassment of respiration. The edema after one hour was decidedly more marked than that of the control and remained so during the time of observation. The next day the lesions were much more pronounced than those of the control.

Rabbit 154. 1660 grams. 8 cc. NaCN had but slight symptoms. The reaction was even more intense than that of no. 153.

Rabbit 156. 1320 grams. 6 cc. NaCN, had but slight symptoms. Reaction exceeded that of control. Next day lesion much more pronounced than that of control.

Rabbit 158. 850 grams. 6.5 cc. NaCN, had but slight symptoms. Reaction even more pronounced than of no. 156.

Rabbit 159. 1270 grams. 6 cc. NaCN, had but very slight symptoms. The reaction throughout was about like control.

Rabbit 170. 1930 grams. Received first 10 cc. NaCN without any symptoms, then 4 cc. more were injected resulting in convulsions and marked dyspnoea, rapid recovery. The reaction was certainly not less intense than that of the control and perhaps a little more.

Rabbit 172. 2390 grams. 16 cc. NaCN. Convulsions, marked dyspnoea, rapid recovery. The reaction was throughout less intense than that of the control.

In four of the seven experiments the reaction of the animals injected intravenously with NaCN was decidedly more intense than the reaction of their control animals. These animals showed but slight constitutional symptoms such as some embarrassment of respiration and some excitement. In two animals (nos. 159 and 170) the reaction did not differ markedly from their controls;—no. 159 had but very slight symptoms, no. 170 on the contrary showed very marked symptoms, as marked as no. 172 where an inhibition was noted. Taking in consideration the difficulty of finding the proper dosage of this most potent drug for each individual animal it is evident that the intravenous injection of NaCN is capable of intensifying the conjunctival mustard oil reaction of the rabbit. A few experiments were made to see whether it were possible to overcome the inhibition of the mustard oil reaction due to sodium iodoxybenzoate by means of HCN. The solution used contained 1.7 per cent HCN. It is to be noted that the dosage of sodium iodoxybenzoate employed here never failed to produce a most marked lasting inhibition.

Rabbit 131. 1720 grams. Received 17 cc. $\frac{M}{20}$ sodium iodoxybenzoate intravenously, then 1 drop HCN in one eye and 1 drop of mustard oil in the other eye. After two hours there was a considerable edema.

Rabbit 133. 1400 grams. Received 12 cc. $\frac{M}{20}$ sodium iodoxybenzoate intravenously then 1 drop HCN in one eye and 1 drop mustard oil in the other eye. Two and one-half hours later there was considerable edema progressing to marked edema.

Rabbit 134. 1380 grams. Control. 12 cc. $\frac{M}{20}$ sodium iodoxybenzoate, then mustard oil in eye. After thirty minutes slight edema, hardly progressing any further.

Rabbit 135. 1380 grams. Received 12 cc. $\frac{M}{20}$ sodium iodoxybenzoate intravenously then 1 drop HCN in one eye and 1 drop mustard oil in the other eye. One and one-half hours later there was a slight edema, the animal received another drop HCN. Thirty minutes later there was considerable edema. Thirty minutes later received another drop HCN. One-half hour later there was not much increase of the edema. The next morning there was considerable edema and secretion, hyperemia and opacity of cornea.

Rabbit 136. Control 1400 grams. Received 12 cc. $\frac{M}{20}$ sodium iodoxybenzoate, intravenously, and a drop of mustard oil in the eye. Thirty minutes later there was a slight edema which had perhaps increased a little seven hours after the injection. The next morning there was some injection, slight edema and the cornea was clear.

Rabbit 81. 1980 grams. Received 13 cc. sodium iodoxybenzoate solution, followed by HCN and mustard oil, it showed a marked edema after two hours.

Rabbit 68. 1040 grams. 10 cc. sodium iodoxybenzoate. HCN and mustard oil. In this animal the result was less pronounced.

Seven other animals treated in the same manner died.

The results indicate that the inhibition caused by the intravenous injection of sodium iodoxy-benzoate may be overcome to a certain extent by means of hydrocyanic acid.

GENERAL DISCUSSION

The data presented show that the sodium iodoxy- and iodosobenzoates exhibit their inhibiting effect on the inflammatory edema produced by mustard oil applied to the conjunctiva of the rabbit. This inhibition is just as pronounced as when the oil was administered intracutaneously, as in previously recorded experiments. The fact that various other substances containing chemically active oxygen also exercised such an inhibiting in-

fluence, even if not to the same degree, lends strong support to the conception that the chemically active oxygen is concerned in some way in the activity.

Some of the results obtained by Amberg and Knox (1) pointed rather strongly to the possibility that the sodium iodoxybenzoate may unfold its activity by virtue of an influence exercised on the tissue where the inflammatory stimulus was applied. In these experiments the inflammation was produced by the injection of horse serum intracutaneously in rabbits sensitized to horse serum. Diluting the serum with a sodium iodoxybenzoate solution offered the great advantage of applying the drug exactly where and when it was wanted. There was an unmistakable inhibiting influence. On the contrary when the serum was diluted with sodium cyanide there was an unmistakable intensification of the reaction. From these results the authors arrived at the hypothesis that measures favoring oxidative processes in the tissues tend to inhibit inflammatory processes, while measures interfering with oxidative processes have the opposite tendency. Speaking in such general terms of oxidative processes in tissues may not be deemed justifiable, taking in consideration the number of oxidations which must take place in the organism, requiring for their accomplishment various conditions. Thus Batelli and Stern (15) showed that the oxidation of alcohol, succinic acid and citric acid by tissues is brought about under very dissimilar conditions. However certain conditions may be assumed to inhibit all oxidations in the body. There are differences in the conduct of iodoso- and iodoxy-benzoic acid, the iodoso-benzoic acid (Grove and Loevenhart (9), Jahn (7)) oxidizing phenolphthalin to phenolphthalein, under proper conditions, while the iodoxy-acid does not. Furthermore the sodium salt of the iodoso-benzoic acid yields oxygen much more readily to the perfused isolated mammalian heart (Loevenhart and Eyster (16)) than does the corresponding salt of iodoxy-benzoic acid. If then a substance with chemically active oxygen like ammonium persulfate does not inhibit the mustard oil inflammation, it does not discredit the view that the inhibitory activity of the other substances is due primarily to their chemi-

cally active oxygen. In the experiments where animals were exposed to an atmosphere with diminished partial pressure of oxygen, the reduction of the oxygen was sufficient to elicit some responses from the organism. Dallwig, Kolls and Loevenhart (17), keeping animals in atmospheres of low partial pressure of oxygen, proved that after a certain length of time there is an increase in the haemoglobin and in the number of red blood corpuscles. David (18) describes a hyperemia of the lungs on shorter exposures to 9 to 10 per cent O_2. Von Terray (19), exposed rabbits and dogs to mixtures of hydrogen and air with varying oxygen tension; the experiments lasted thirty-five to ninety minutes. Coincidently with the first visible changes of respiration at 10.5 per cent O_2 he reports a beginning increase in the excretion of lactic and oxalic acid in the urine. Quite remarkable are his experiments where the animals were kept at about 2.7 per cent O_2 from thirty-four to ninety minutes The signs of oxygen want did not develop immediately but after several minutes. The respiration of Strangonow's (20) animals ceased when the oxygen content of the inspired air fell to 3.5 per cent, a figure agreeing well with those given by previous authors. Loewy (21) in his investigations on man points out that the effect of low oxygen tension depends on the oxygen tension of the alveolar air and that with proper breathing it was possible to sustain an alveolar O_2 tension of 5.5 to 6 per cent when the inspired air contained less than 8 per cent oxygen. When the alveolar air contains less than 5.5 per cent O_2 the oxygen consumption begins to decrease and the respiratory quotient to increase, a sure sign of oxygen want. As a rule the oxygen tension of the alveolar air when the inspired air contained 11.5 to 10 per cent O_2 was 6 to 5 per cent. There was, therefore, in all probability an interference with tissue oxidation in our experiments. In spite of this the mustard oil inflammation was not intensified. These experiments do not support the hypothesis that measures interfering with tissue oxidations tend to favor the inflammatory edema. But at the same time they do not disprove it for various reasons. It must be kept in mind that it may be very difficult to bring about the proper degree or kind of interference with

local oxidations in the tissue here concerned by reducing the oxygen supply to the body as a whole.

The investigations of J. Loeb (22) on the physiological action of oxygen want brought to light some very significant facts. Fructified eggs of ctenolabrus and of the sea urchin were kept in a current of hydrogen washing out the oxygen. When the oxygen had been washed out thoroughly there was no more complete cell division. When the oxygen was not washed out sufficiently cell division took place. We have then to consider the possibility that by the time the oxygen supply to a tissue like the skin is sufficiently reduced to effect the oxidations in question the general effect on the organism may offset any influence the diminished oxidations might have exercised on the inflammation. The experiment with cyanide, by Amberg and Knox, and those given in the preceding pages, show an intensifying effect on the inflammatory edema. Under proper conditions it seems possible to overcome to a certain extent the inhibition produced by sodium iodoxybenzoate with hydrocyanic acid.[5] The great advantage cyanide offers over the methods influencing the oxygen supply to the tissues is that it inhibits oxidations no matter how abundant the oxygen supply (Geppert (23)). This being the case we are at present certainly justified in bringing the intensifying effect of the hydrocyanic acid into correlation with its influence on oxidative processes in the tissues.

The experiments with illuminating gas again illustrate the fact previously noted by Amberg and Knox (1) as well as by Januschke (6) that more or less severely intoxicated animals may not react with a full development of an inflammatory edema. Rarely, quite severely poisoned animals may give surprisingly good reactions.

Both animals which had been bled showed an inhibition. But the rapid withdrawal of about one-third of the total amount of blood must have a rather pronounced and complicated effect on the organism, and the acute reduction of the oxygen carry-

[5] Dr. A. D. Hirschfelder was kind enough to notify us that he also has seen a marked intensifying action of an intravenous injection of NaCN on the conjunctival mustard oil reaction of the rabbit.

ing power of the blood in its effect on the tissues may play a very insignificant rôle. Salant and Wise (24) observed no diminution of the power to oxidize citric acid in animals which had been bled. In the effect of bleeding on inflammation, the tendency of the fluid in the tissues to pass into the vessels may enter in consideration.

We tried to obtain some information with regard to the withdrawal of water from the organism, utilizing the result of Woodyatt (25) with continuous intravenous injections of glucose. A rabbit of 1942 grams received glucose at the rate of 5.1 grams per kilogram an hour with Woodyatt's (8) motor syringe, that is, 57.6 cc. of a 40 per cent solution in water in one hundred and thirty-five minutes. After eighty-two minutes the rabbit voided 49 cc. urine, whereupon mustard oil was instilled in the eye. With the first visible change in respiration the injection was stopped. The rabbit had passed another 54 cc. urine and no more could be expressed from the bladder. The weight had dropped to 1890 grams. The edema became fairly marked during the thirty minutes following the instillation and did not differ very much from that of the control. From this time on it hardly progressed while that of the control proceeded as usual. In a second experiment an animal of 1965 grams having passed in the preceding twenty-four hours about 300 cc. of urine received glucose at the rate of about 6.5 grams per kilogram an hour, the total amount injected being 75.7 cc. of a 40 per cent solution The animal passed urine as follows:

	cc.
After 56 minutes	63
After 71 minutes	45
After 88 minutes	31
After 108 minutes	21
After 112 minutes	20
After 119 minutes	16
After 126 minutes	14
After 136 minutes	14
Total	224
Specific gravity	1.024

The mustard oil was instilled when the injection had been going eighty-one minutes and there was hardly any edema, while the experiments lasted. About the time of the last urination changes in the respiration became apparent and when the injection was stopped four minutes later, the animals was sick, shivering intensely and died fifty-one minutes later. But until nearly the end of the injection this animal did not show any symptoms whatever. In these experiments the dehydration of the organism is probably not the only factor bringing about the inhibition, but that it does play a considerable part may be regarded as fairly certain when we take in consideration that Sansum and Wilder (26) were able to reduce the intraocular pressure in cases of glaucoma very considerably by means of such glucose injections leading to a considerable diuresis.

A number of factors exercise an influence on the production of edemas.[6] The data at our command emphasize the possibility that the condition of the tissues brought about by the injury leading to the inflammation plays a rôle in the production of the inflammatory edema. Furthermore within certain limits an influence on tissue oxidations seems to determine an inhibition or intensification of the response to inflammatory stimuli. Such an effect could be most readily explained on the basis of the theory of Fischer (27) as an influence on the hydration of the tissue colloids. Some obstacles such as the results of Hirschfelder (28) make it impossible to accept this explanation unconditionally. Hirschfelder dropped mustard oil in the conjunctival sac of rabbits, cut out the lids and placed them in Ringer's solution or rabbit's serum, without noticing any swelling.[7]

Januschke (6) studied the effect of various substances on the mustard oil swelling of the rabbit's conjunctiva and on the basis of his results and those of others he attributes the inhibiting effect noted to an influence on an apparatus of inflammation,

[6] See for instance, Wells Chemical Pathology, 1917, Chapter XII, Edema.

[7] There is another possibility of explaining the inhibiting action of the substances containing chemically active oxygen on the one hand and the intensifying one of cyanide on the other by the assumption of an influence on the permeability of the vessel walls. Hereby again an influence on the hydration of the colloids of the vessel walls has to be taken into account.

or at least of exudation of the conjunctiva. This apparatus consists of sensory nerve endings, nerve fibers and blood vessels. Exclusion of the sensory nerve endings by degeneration or by drugs such as narcotics or analgesics inhibit the mustard oil reaction. Substances like calcium and epinephrin are supposed to act on the vessels, possibly by an influence on their permeability. In this scheme of Januschke the beautiful experiment of Bruce (4) is of great importance. The destruction of the ophthalmic branch of the third nerve, when sufficient time for the degeneration of the peripheral part was allowed, inhibited the mustard oil reaction of the conjunctiva completely. Stevenson and Reid (29) were unable to confirm the results of Bruce, neither did their clinical experiments, applying cantharides plaster to anesthetic areas in which the sensory nerve endings have degenerated, support the view that the sensory nerve endings are necessarily concerned in the inflammatory reaction. It seems that an inflammation can occur when the sensory nerve endings are excluded from the field of action. With the exclusion of the sensory nerve endings there remains of the exudation apparatus of Januschke only the vessels. That is we return to the conceptions of Cohnheim (30) who refers the inflammatory edema to an injury of the vessel walls. It is of particular interest to us here that Cohnheim regards this injury as due primarily to a lack of oxygen. The conception toward which we lean is really still older, going back to Virchow (31). It is, that reactions set in motion in the tissues bearing the first brunt of the injury participate in the production of the inflammatory edema.

Vieles ist, wie wir sehen, nicht ein aus den gefässen durch den Blutdruck hervorgepresstes, also passives Exudat, sondern vielmehr, wenn ich mich so ausdrücken soll, Educt oder Extract aus den Gefässen in Folge der Thätigkeit, der activen Anziehung der Gewebselemente selbst.

The chief objection of Cohnheim was that such attractions were unknown to physiology, an objection having lost its force in the course of time.

CONCLUSIONS

The intravenous injection of the sodium salts of o-iodoso- and o-iodoxy-benzoic acid inhibits the mustard oil reaction of the rabbit conjunctiva as well as that of the skin. No other visible changes in the organism accompany this inhibiting action. Other substances containing chemically active oxygen such as the sodium salts of diphthalic peroxide, iodic and periodic-acids have an inhibiting action on the mustard oil inflammation.

It seems reasonable to ascribe the inhibiting action of these substances to the chemically active oxygen.

Exposure of rabbits to atmospheres with reduced partial pressure of oxygen did not have any marked effect on the intensity of the intracutaneous mustard oil reaction.

Intravenous injections of NaCN seem capable of intensifying the conjunctival mustard oil reaction under proper conditions.

Within certain limits an influence on tissue oxidations seems to determine an inhibition or intensification of the response to inflammatory stimuli.

It seems possible to alter the character of the conjunctival mustard oil reaction by intravenous injections of substances, such as sodium hypochlorite.

Irritation produced elsewhere in the organism, as by intraperitoneal injections of kaolin, animal charcoal, mustard oil, may exercise an inhibiting influence on the conjunctival mustard oil reaction of the rabbit.

REFERENCES

(1) Amberg and Knox: Jour. Pharm. and Exper. Therap., iii, 1912, p. 223; J. Am. Med. Assoc., lix, 1912, p. 1598.
(2) Amberg: Zeitschr. f. die gesamte experimentelle Medizin, ii, 1913, p. 19.
(3) Arkin: Journ. of Infec. Dis., xvi, 1915, p. 349.
(4) Bruce: Archiv. f. exper. Pathol. u. Pharm., lxiii, 1910, p. 424.
(5) Chiari und Januschke: Archiv. f. exper. Pathol. u. Pharm., lxv, 1911, p. 120.
(6) Januschke: Wiener Klin. Woch., xxvi, 1913, no. 22.
(7) Jahn. Archiv. f. exper. Pathol. u. Pharm., lxxvi, 1914, p. 16.
(8) Woodyatt: Jour. of Biolog. Chem., xxix, 1917, p. 355.
(9) Grove and Loevenhart: Journ. Pharm. and Exper. Therap., i, 1909–1910, p. 289.

(10) Bayer und Villiger: Ber. d. deutsch. chem. Gesellsch., xxxiv, 1901, p. 762.
(11) Loevenhart: Therap. Monat., xix, 1905, p. 426.
(12) Winternitz, R.: Archiv. f. exper. Pathol. u. Pharm., xlvi, 1901, p. 153.
(13) Kolls and Loevenhart: Am. Jour. Physiol., xxxix, 1915, p. 67.
(14) Henderson, Y.: J. Am. Med. Assoc., lxvii, 1916, p. 580.
(15) Battelli und Stern: Biochem. Zeitschr., xxxi, 1911, p. 478.
(16) Loevenhart and Eyster: Jour. Pharm. and Exper. Therap., v, 1913–1914, p. 21.
(17) Dallwig, Kolls and Loevenhart: Am. Journ. Physiol., xxxix, 1915, p. 77.
(18) David: Zeitschr. f. Klin. Medizin, lxxiv, 1912, p. 404.
(19) Von Terray: Arch. f. d. ges. Physiol., lxv, 1897, p. 393.
(20) Stronganow: Arch. f. d. ges. Physiol., xii, 1876, p. 18.
(21) Loewy: Untersuchungen über die Respiration, etc. Berlin, 1895.
(22) Loeb, J.: Arch. f. d. ges. Physiol., lxii, 1896, p. 249.
(23) Geppert: Zeitschr. f. Klin. Med., xv, 1889, p. 208, 307.
(24) Salant and Wise: Jour. Biolog. Chem., xxviii, 1916, p. 27.
(25) Woodyatt, Sansum and Wilder: J. Am. Med. Assoc., lxv, 1915, p. 2067.
(26) Sansum: J. Am. Med. Assoc., lviii, 1917, p. 1885.
(27) Fischer, M. H.: Oedema and Nephritis. New York, 1915.
(28) Hirschfelder: Jour. Am. Med. Assoc., lxvii, 1916, p. 1891.
(29) Stevenson and Reid: Johns Hopkins Hosp. Bull., xxvi, 1915, p. 21.
(30) Cohnheim: Vorlesungen über allgemeine Pathologie, 2d ed. Berlin, 1882.
(31) Virchow: Cellular pathologie, 3d edition. Berlin, 1892, p. 363.

FOCAL LESIONS PRODUCED IN THE RABBIT BY COLON BACILLI ISOLATED FROM PYELOCYSTITIS CASES *

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The mode of infection in pyelocystitis of the infant and child is still far from settled. Much evidence has been brought forward for each of the three routes of infection, the hematogenous, the lymphogenous, and the ascending. In each case the evidence adduced is generalized, and the route of infection made probable by the investigator's observation is claimed as the only possible one. De facto, these observations do not at all preclude the other routes of infection. When we examine the facts as they are at the present time we realize that we do not know what portion of the urinary tract is infected in the condition that is usually termed pyelocystitis. We do know that those cases that come to necropsy usually show some involvement of the kidney pelvis, but that does not prove that a great many of the cases that we see with pus in the catheterized specimen of bladder urine are not merely cases of cystitis. Until these cases are studied more carefully, we must leave this an open question.

Recently the hematogenous mode of infection has been given a great deal of prominence by the work of Cabot and Crabtree,[1] Kowitz[2] and others, who regard the hematogenous route as the only mode of infection. Smith[3] comes to the same conclusion and meets the main argument against the hematogenous mode of infection, the predominance of cases in the female, by assuming that "many cases in the female, accounting for the greater number in this sex as compared with the males, arise from bacteria entering the blood, often via the

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* From the Otho S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital.

* Read at the meeting of the American Pediatric Society, White Sulphur Springs, W. Va., May 28, 1917.

1. Cabot and Crabtree: Etiology and Pathology of Nontuberculous Renal Infections, Surg., Gyn. and Obst., 1916, p. 495.

2. Kowitz: Ueber bacterielle Erkrankungen der Harnorgane im Säuglingsalter, Jahrb. f. Kinderh., 1915, p. 309.

3. Smith, R. M.: Recent Contributions to the Study of Pyelitis in Infancy, Am. Jour. Dis. Child., 1916, **12**, 273.

lymphatics from the urethra, vulva or vagina." He does not give any evidence, however, in support of this theory, and merely assumes that this may take place.

It seemed to us rather important, therefore, to determine whether or not pyelocystitis could be produced by the hematogenous route. The literature in this connection is very meager. Barlow[4] injected animals intravenously with colon bacilli, without any very definite results. More recently Rosenow's[5] work in the selective localization of bacteria, especially the streptococcus, has called attention to the tendency of bacteria to settle in definite organs. Although his publications do not deal with the colon bacillus alone, he shows that when colon bacilli are injected with streptococci, the tendency is to get lesions in the appendix, gallbladder and intestines. In a series of 31 animals, 26 had lesions in the appendix, 4 of the gallbladder and 13 of the intestine; as compared with only 4 positive findings in a series of 59 animals injected with the streptococci alone. When the colon bacillus alone was injected lesions were very infrequent. This symbiosis is rather interesting when taken in connection with our experiments, in which a mixture of colon bacilli and pneumococci was injected. As in the work of Rosenow, our takes in the kidney were very much more frequent when a mixture of organisms was injected. In order to bring out this difference more strikingly we have kept the two series separate. Series 1 consists of eight different strains of *Bacillus coli* injected intravenously into sixty-six rabbits. Series 2 consists of a mixture of *Bacillus coli* and the pneumococcus injected intravenously into eleven animals.

TECHNIC

The technic employed in the experiments was as follows:

For twenty-four hours before injection the animals were put on a bread and water diet so as to have an acid urine at the time of injection. In every instance the urine was examined before injection for pus cells and albumin. The animals were injected into the ear vein with 0.5 to 1 c.c. of a bacterial suspension, made by centrifuging 15 c.c. of a twenty-four-hour broth culture, and suspending the bacilli in 5 c.c. of salt solution. As indicated in the protocols, the injections were single or repeated.

Series 1: In this series of 66 animals, 26 showed focal lesions of one kind or another. The organs were affected as follows: Kidneys 11 times, cecum 7 times, gallbladder 7 times, appendix 5 times, and stomach 4 times; in addition, there were single instances of hemorrhagic infarction of the descending colon, ulcerative colitis, hemor-

4. Barlow: Cystitis, Arch. f. Dermat. u. Syph., 1893.

5. Rosenow: The Bacteriology of Appendicitis and Its Production by Intravenous Injection of Streptococci and Colon Bacilli, Jour. Infect. Dis., 1915, p. 240.

rhagic enteritis, and duodenal hemorrhage. In Table 2 the positive results have been tabulated.

Taking up the different organs in the order of the table, we find that the appendix was affected in five instances; in two of these there was a typical acute purulent hemorrhagic appendicitis, with gangrene near the base of the appendix in the one. This appendix is shown in Figure 1. In the three other instances the appendix showed considerable subserous edema and numerous small hemorrhages.

The stomach was affected in four instances. In two instances there was an unusual number of small hemorrhages in the mucosa, and in one instance there was a phlegmonous gastritis, most marked at the

TABLE 1.—Characteristics of Organisms Used in Series 1

Organism	Motility	Indol	Gelatin	Litmus Milk	Dextrose	Lactose	Saccharose
B. Coli Communis Injected in:							
B. Series C.....	+	+	0	Acid and Coag.	+	+	0
P. Series H.....	+	+	0	"	+	+	0
S. Series H.....	+	+	0	"	+	+	0
B. Coli Communior Injected in:							
J. P. Series C, D and E	+	+	0	"	+	+	+
C. Series L.....	+	+	0	"	+	+	+
L. Series C.....	+	+	0	"	+	+	+
B. Series F and G	+	+	0	"	+	+	+
B. Paracolon Injected in:							
E. Series C.....	+	+	0	Acid; alk. after 48 hours	+	0	0
L. Series H.....	+	+	0	"	+	+	0
Organisms used in Series 2							
B. Coli Communior and Pneumococcus	+	+	0	Acid and Coag.	+	+	+

pyloric end and involving almost one-half of the stomach. The stomach wall was immensely thickened and edematous, especially the muscular coats, and microscopic sections showed diffuse leukocytic infiltration and edema. The fourth instance was a typical gastric ulcer, with a markedly edematous margin, and numerous small hemorrhages in the base. Cultures from the surface of the ulcer and deep down in the tissues yielded only a colon bacillus. This organism was reinjected into a series of animals without producing an ulcer in any one of them.

The gallbladder was affected seven times. The lesions varied from a slight thickening, with minute hemorrhages, to a purulent cholecystitis with fibropurulent gluing together of the stomach, colon and liver. The relatively large number of rabbits that showed coccidiosis

of the gallbladder may account for the number of gallbladder infections that were produced.

The cecum was involved in seven instances. The lesion was a peculiar edematous induration that involved only a few centimeters of the cecum or extended over from 12 to 15 cm. of the entire wall. Figure 2 shows very well the peculiar edematous swelling of the entire wall and also the peculiar hemorrhagic necroses at the tops of the folds

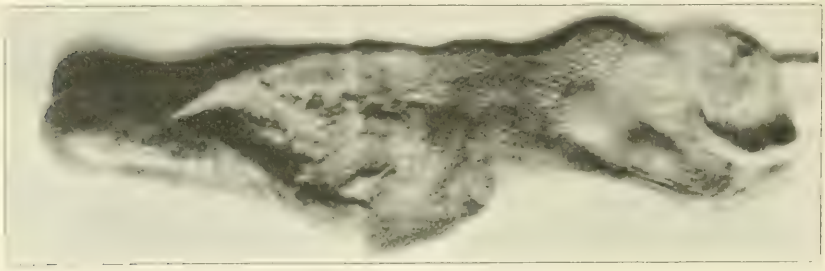

Fig. 1.—Hemorrhagic appendicitis.

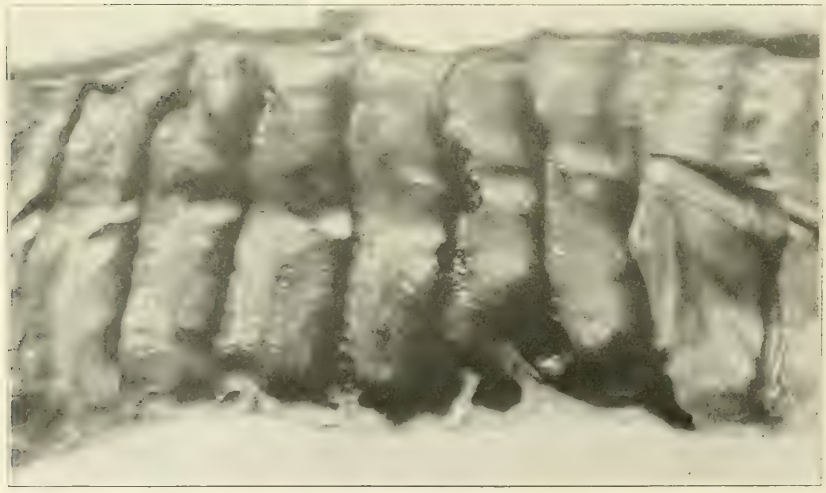

Fig. 2.—Edematous induration of the cecum.

of the mucous membrane. In several instances a diphtheritic exudate could be seen on the tips of the folds. In some the edema was the most marked condition; in others the hemorrhagic condition of the mucous membrane. On section, the lesions proved to be a tremendous inflammatory edema with necrosis of a large portion of the mucous membrane at the tips of the folds. There was also to be seen an inflammatory exudate on the peritoneal surface.

TABLE 2.—Data of Experiments in Series 1

No.	Animal	Date	Source and Amount Injected, C.c.	Days Until Death	Appendix	Stomach	Gallbladder	Cecum	Kidney				Urine			Remarks
									Hemorrhage	Cortical Abs.	Medullary Abs.	Pyelitis	Albumin	Pus	Culture Colon	
1	R 1X....	5/23 5/24	½ R1V.. ½.....	D 4	+	+	0	0	0	0	+	0	+	0	+	Phlegmonous gastritis; pel. culture, gram negative bacillus
2	R X.....	5/23 5/24	½ R1V.. ½.....	D 3	0	0	0	+	0	0	+	0	+	..	+	Pelvis culture, gram negative bacillus
3	R X111..	5/27	1 RX....	D 1	0	0	0	+	0	+	+	0	+	..	..	Necropsy 24 hours after death
4	R 2D....	5/30 5/31	⅕..... 1 R1X...	D 3	0	0	0	+	0	+	0	0	..	0	+	
5	R 3D....	5/30 5/31	⅕ R1X.. 1.....	S 3	0	+	0	0	0	0	0	0	..	..	0	Stomach ulcer; pure culture, gram negative bacillus from base of ulcer
6	R 4D	5/30 5/31	½ R1X.. 1.....	S 3	0	0	+	0	0	0	0	0	..	..	..	Culture of gallbladder, pure gram negative bacillus
7	R 5D....	5/30 5/31	1/10 R1V ½ app...	S 4	0	0	+	0	0	0	0	0	..	0	+	Culture of gallbladder, pure gram negative bacillus
8	R 6D....	5/30 5/31	⅕ app... ½...	D 2	0	0	0	0	0	0	0	0	+	0	..	Hemorrhagic infarct. of desc. colon; kidney culture, pure gram negative bacillus
9	R 10D...	5/30 5/31	⅕ app... ½.....	D 2	0	0	0	+	0	0	0	0	..	..	..	Peritonitis; hemorrhage in bladder
10	R 11D...	5/30 5/31	⅕ app... 1.....	S 4	0	0	0	0	0	0	0	0	+	+	+	Hemorrhage in bladder
11	R 1E....	6/ 3	½ R4D..	S 3	0	0	+	0	0	0	0	0	..	..	..	
12	R 2E....	6/ 3	1 R4D...	D 3	0	0	+	0	+	0	0	0	..	..	..	
13	R 4E....	6/ 3	½ R3D..	D 2	0	0	0	+	0	0	0	0	..	..	..	
14	R 1H....	6/21	1 N. L...	D 2	+	0	0	0	+	0	0	0	..	..	0	
15	R 2H....	6/21	1 N. L...	D 2	+	0	0	0	0	0	0	0	..	..	..	Peritonitis
16	R 5H....	6/22	1..... Perin....	Dying D 1	+	0	0	0	0	0	0	0	0	0	+	Marked hemorrhage into duodenum
17	R 9H....	6/23	1.....	D 1	+	0	0	+	0	0	0	0	0	0	..	Hemorrhagic enteritis
18	R 11H...	6/26	Sullivan Bl. of... R8H.....	Dying 1 S	0	+	0	0	0	0	0	0	0	0	0	Enteritis; hemorrhage in duodenum
19	R 2K....	6/28	Bl. R7G. 1.....	S 5	0	0	0	0	0	0	0	+	+	R O L +	+	Pus from left, clear urine from right ureter
20	R 6K....	6/28	Bl. R7G. 1.....	S 5	0	0	+	0	0	0	0	+	+	R + L O	+	Pus from right ureter; clear urine from left
21	R 7K....	6/28	Bl. R7G. ½.....	D 2	0	0	0	+	0	+	+	0	0	0	+	Peritonitis
22	R 15K...	7/ 4	Bl. R2K. 1.....	D 3	0	0	0	0	0	0	0	0	+	+	..	Ulcerative vaginitis
23	R 17K...	7/ 4	Bl. R2K. 1.....	D 4	0	0	+	0	0	0	0	0	..	..	..	
24	R 1L....	7/12	3.....	S 4	0	0	+	0	0	0	0	0	0	0	..	

The kidney showed focal lesions eleven times; inasmuch as three of these were only hemorrhages of very slight extent, in two of the instances only seen microscopically, it must be considered doubtful whether they have any definite relationship to the colon bacillus injections. The remaining eight animals showed kidney lesions as follows: three had cortical abscesses, three had medullary abscesses, and two had a unilateral pyelitis.

Cortical Abscesses. — In one rabbit the cortical abscesses were apparently of very recent origin and had not spread along the tubules, down into the medulla. In the other two, the abscesses had involved the medulla also. In one of these animals the abscesses were visible

Fig. 3.—Cortical abscesses.

to the naked eye as small, opaque, yellowish nodules in the surface of the kidney, which on section could be seen extending down into the medulla, as seen in Figure 3. In the other of these two animals, the lesions were only discovered in the microscopic examination of the kidney section. The abscesses in the cortex were very minute, involving one or two glomeruli, and those in the medulla showed a tubule or two filled with pus, about which there was some infiltration. The pelves in both of these cases were normal in the sections examined.

Medullary Abscesses.—Two of the three cases showed practically the same condition, a practically normal, reddish-brown kidney externally, and on section, small, yellowish, opaque nodules in the medullary portion of the kidney. These proved to be minute abscesses in which numerous bacilli could be demonstrated. The third rabbit showed a very different picture; the kidney was very much swollen and slightly discolored by postmortem changes, but showed in spite of that, very

extensive abscesses in the medulla; the cortex as far as abscess foundation went was practically intact. The medulla was fairly studded with abscesses extending down along the tubules as seen in Figure 4.

Pyelitis.—Of the sixty-six animals injected, only two showed a typical pyelitis, and in both instances the pyelitis was unilateral. In both instances pus could be expressed from one ureter and clear urine from the other; and on section of the kidney pus welled out of the pelvis in one and a small amount of clear urine out of the other. Histologically, the kidneys of the unaffected and affected side were practically identical, except that in the one the pelvis was filled with pus; in the other not. Changes in the wall of the pelvis were manifest only in an excessive number of polymorphonuclear cells in certain areas.

Fig. 4.—Medullary abscesses.

The question naturally arises at this point as to what the primary lesion is in pyelitis, and why should one get a unilateral pyelitis in these two cases? It emphasizes the fact that unilateral pyelitis does not necessarily point toward the lymphatic mode of infection, but that bacteria may lodge in one kidney and grow, and not in the other even when larger doses are given intravenously than could ever be conceived of in human pathology.

Series 2: Over against Series 1 we wish briefly to summarize experiments of Series 2 (Table 3), consisting of eleven animals which were injected with a mixed culture of pneumococci and colon bacilli isolated from a severe case of bronchitis and pyelitis, in which on catheterization only a glass catheter full of practically pure pus could be obtained just before death. Of the eleven animals injected, six showed focal lesions of the kidney; three had a pyelitis; one had cortical abscesses, and two had diffuse hemorrhages in the cortex.

TABLE 3.—Data of Experiments in Series 2

No	Animal	Date	Source and Amount Injected, C.c.	Days Until Death	Appendix	Stomach	Gall-bladder	Cecum	Kidney				Urine			Remarks
									Hemorrhage	Cortical Abs.	Medullary Abs.	Pyelitis	Albumin	Pus	Culture Colon	
1	R 2F...	6/ 9	S & C.. 1.....	S 8	0	0	0	..	..	..	..	+	+	-	C + P +	
2	R 3F...	6/ 9	S & C... $\frac{1}{2}$.....	S 6	..	..	+	..	..	+	..	..	+	+	C + P +	Pericarditis; culture, pure pneumococcus; pneum. and colon from pelvis of both kidneys
3	R 4F....	6/ 9	S & C... 1 B.....	S 8	0	0	0	0	0	0	0	+	+	+	C + P -	
4	R 39G...	6/17	S & C... 1 Kid. of R3F	D 2	+	0	0	+	+	0	0	0	.	0	C +	Pure culture B. coli from appendix; culture from kidney cortex negative
5	R 19G...	6/19	S & C... 1 Kid. of R3F	S 2	0	+	0	0	+	0	0	0	+	0	0	
7	R 79G...	6 20	S & C... 1 Kid. of R3F	Dying 7 S	0	0	0	0	0	0	0	+	+	+	C +	
					*1	*1	*1	*1	*2	*1	*0	*3	*5	*4	..	

* Totals.

The hemorrhages in the one were very severe, as over two-thirds of the kidney surface was hemorrhagic; in the other the hemorrhages, though less severe, were more marked than in any of the rabbits injected with colon bacilli alone.

Cortical Abscesses.—In the one case with cortical abscesses the lesion was very widespread. There were large, yellow, raised areas on the surface of the kidney that extended down in wedge-shaped areas to the tips of the papilla.

Pyelitis.—Of the three cases of pyelitis, one must be put in the doubtful column, because the diagnosis of pyelitis rested on the finding of masses of pus cells in the pelvis of the kidney as well as in the cross-section of the ureter histologically, without having found any in the specimens of urine examined, and without any findings macroscopically at necropsy. In the second there were pus cells in the urine, but a practically empty pelvis at necropsy. Histologically, however, there were seen in section definite areas of leukocytic infiltration in the pelvis wall.

In the third case the kidney substance was normal in both, except that the medulla looked somewhat edematous; the pelvis in both kidneys exuded thick creamy pus when opened up. The pelvic mucous membrane was practically intact in the sections studied, but the lumen of the pelvis was filled with a solid mass of polymorphonuclear leukocytes, red blood cells and mononuclear cells.

These three cases emphasize again the difficulty of a study of pyelitis. The first two would have been simply overlooked if sections had not been studied, and it is readily conceivable that a section taken in another portion of the kidney would not have showed these changes.

As controls for these experiments, we injected four different strains of colon bacilli isolated from the intestinal canal of infants suffering from slight intestinal disturbances. Three animals were injected with each strain. Of the twelve injected, in only one animal were there any local findings. This was a purulent appendicitis. The urinary examinations in all the animals were entirely negative; not a single animal showed pus or albumin in any of the examinations made.

The results of the experiments were a considerable surprise. We had anticipated a much larger series of takes in the kidney than were obtained. In Series 1, of the sixty-six animals injected, only eight, about 12 per cent., showed lesions that could be attributed to the *Bacillus coli.* In the same series the appendix was involved 5 times, the stomach 4 times, the gallbladder and the cecum each 7 times. It is thus evident that of the eight organisms used, none showed a special tendency to localize in the kidney, and when it did localize the focal lesions were just as likely to be in other organs, especially the gastro-

intestinal tract. The most characteristic lesion of the colon bacillus appeared to be the edematous infiltration of the cecum. It would seem that the rabbit in almost 90 per cent. of instances can excrete colon bacilli through the kidney without any harm to the kidney. If we compare this with the results obtained by injecting a mixture of pneumococci and colon bacilli, we find that the kidney is more frequently affected. In six out of eleven rabbits, or in 60 per cent., the mixture caused definite lesions in the kidney. This increase of focal lesions corresponds closely to the difference in appendiceal localization described by Rosenow, when a mixture of colon bacilli and streptococci was injected. The question naturally arises whether in human pyelocystitis there is frequently a double infection. The second organism may be easily overlooked, because cultures are usually not made early when both organisms would most likely be present, and if present the colon bacillus tends to overgrow the other. In two cases of hematogenous streptococcus pyelitis in the rabbit we could observe that the colon bacillus made its appearance later, and still later completely displaced the streptococcus.

CONCLUSIONS

1. It is possible to produce typical pyelocystitis in the rabbit by the intravenous injection of colon bacilli isolated from human cases.

2. If a mixture of organisms is injected, the relative number of kidney infections can be greatly increased.

PROTOCOLS

Rabbit 1X.—Weight, 1 pound 3¼ ounces.

May 23, 1916. Urine dark yellow; amorphous precipitate; alkaline. Microscopic: amorphous precipitate; 0.5 c.c. bladder culture of Rabbit 1V injected intravenously; bread diet.

May 24. Urine acid; negative; 0.25 c.c. bladder culture Rabbit 1V reinjected intravenously.

May 25. Urine dark yellow, thick, slightly cloudy, strongly acid, slight trace of albumin. Microscopic: few cells; no precipitate; no bacteria. Stained urine, no bacteria.

May 26. Urine yellow, cloudy, thick, acid; albumin +.

May 27. Rabbit died.

Necropsy.—On opening the peritoneum there is a fibrinous exudate over the upper portion of the abdominal cavity. The stomach is deeply injected and also the ascending portion of the duodenum. The appendix is about the size of a little finger, firm, of yellowish opaque color, deeply injected, in one area greenish black and necrotic looking. On opening the appendix the wall is thickened and hemorrhagic. The lumen is filled with hemorrhagic, yellowish-brown detritus. The duodenum on opening shows areas in which there have been numerous hemorrhages. The stomach wall is intensely thickened and edematous in the pyloric region, less markedly so in the cardiac end. The kidneys are of a brownish-red color, the capsule strips readily. There is nothing unusual externally. On section midway between cortex and papilla there are numerous fine yellowish streaks and spots about 1 mm. across.

May 29. All cultures were of an atypical colon bacillus. Bladder urine acid; no excess of cells; albumin ++.

Histology.—Section 5: The stomach shows an enormous edematous swelling of the submucosa. Most of the cells in the edematous area are lymphocytes. There are numerous hemorrhages into the muscularis.

Section 3: The pancreas and duodenum show marked hemorrhages into the submucosa; also in the muscle and parenchyma. The tissues are well preserved.

Section 4: The liver is negative except for cloudy swelling.

Section 5: Appendix. There is no mucosa left. There is massive infiltration of the lymphatic tissue, with a large area of necrosis and hemorrhages. The muscle layer is densely infiltrated.

Section 6: Kidney. There are miliary abscesses in the central portion of the medullary portion. The medulla in large area is densely infiltrated with lymphocytes, separating the collecting tubules. The cortex shows only slight swelling. There are no hemorrhages.

Rabbit X.—Weight, 1 pound 3 ounces.

May 23. Urine clear; slight amount of phosphate precipitate. Microscopic: no cells; no albumin; alkaline; 0.5 c.c. bladder culture Rabbit 1V injected intravenously; bread diet.

May 24. Urine acid, negative. Reinjected with 0.5 c.c. bladder culture from Rabbit 1V.

May 25. Urine bright yellow, clear, acid; albumin, a trace. Microscopic: no cells; slight precipitate; no bacteria; stained urine, no bacteria.

May 26. Died.

Necropsy.—No distention of bladder. Bladder and pelvis of right kidney were cultured. The kidneys are of reddish-brown color, with no external changes. The capsule strips readily. On section the cortex is slightly more opaque than usual. There is no mottling. The pyramids, beginning about the middle, are studded with small yellowish opaque dots and streaks extending down toward the papilla; the pelvis is everywhere smooth. No injection or hemorrhage is to be made out in either kidney. The lungs and heart were negative. The other organs were negative.

May 29. Pelvis of kidney and bladder yielded a pure culture of colon bacillus. (J. P.)

Rabbit XIII.—May 27. Bladder culture from Rabbit X injected intravenously. Urine acid, clear, pale, straw color; albumin, 0.

May 28. Died.

Necropsy.—A loop of colon is covered with tags of purulent exudate, markedly injected, and a hemorrhage on the surface covers an area 2 cm. in diameter. Appendix normal; heart and lungs, and kidneys negative on macroscopic examination.

Histology.—Kidney: There are miliary abscesses in the cortex involving several glomeruli; glomeruli filled with pus cells. In the tubules of the medulla near the tip of the pyramid are several tubules distended with a purulent exudate. One has extended to the tissue between the tubules. In the same portions of the kidney there are tubules filled with masses of bacilli. The pelvis of the kidney shows no change. Colon: The tissue is infiltrated with red blood corpuscles and almost entirely necrotic; only the muscular layer still shows nuclear stain.

Rabbit 2D.—Weight, 15 ounces.

May 30. Cultures of kidney of Rabbit 1X, 0.2 c.c. injected intravenously. Urine entirely negative on examination.

May 31. Reinjected, 1 c.c.

June 2. Died.

Necropsy.—On opening the peritoneal cavity the cecum just at the ileocecal

valve is thickened and hemorrhagic; on opening up this portion of the cecum it is seen to be edematous. The wall is considerably infiltrated, the rugae thickened and numerous hemorrhages on the mucous surface. The kidneys are smooth, of a brownish-red color, and show just under the capsule numerous yellow, opaque nodules about 5 mm. in diameter. The bladder shows some hyperemia of the walls. The gallbladder lies free, studded with white areas similar to Rabbit 4D but not so marked. The other organs are normal.

The urine shows casts and a large number of epithelial cells. Cultures from bladder and cortex of kidney give a colon bacillus.

Histology.—Cecum: There is practically a phlegmon of the entire wall. The submucosa is rich with purulent exudate; mucosa hemorrhagic; only few acini of epithelium remain; muscularis infiltrated with pus cells.

Rabbit 3D.—Weight, 15½ ounces.

May 30. One c.c. culture from kidney of Rabbit 1X injected.

May 31. One c.c. reinjected.

June 2. Sacrificed.

Necropsy.—The abdominal cavity is entirely negative, the cecum and appendix are normal. The bladder is distended. The stomach and duodenum are normal externally. On opening the stomach the cut passes through an ulcer, measuring about 4 mm. across. The ulcer is sharply defined, with a yellowish base, in which are numerous hemorrhages; the edges are thickened, markedly edematous and somewhat wavy. The base is on the muscular layer. Cultures from base and edematous area around the ulcer gave a gram-negative bacillus, an atypical colon bacillus, as was the original J. P. organism. The bladder culture was negative.

Histology.—Section of Kidney: Marked cloudy swelling and vacuolar degeneration of parenchyma. Medulla and pelvis normal. In some areas, apparent necrosis of epithelium.

Stomach: The ulcer shows very intense reaction about it; very marked edema and polymorphonuclear infiltration. In the ulcer base there are a considerable number of red blood corpuscles. The serosa is intact, although there is considerable infiltration just below it.

Rabbit 4D.—Weight, 15½ ounces.

May 30. One-half c.c. of culture from kidney of Rabbit 1X injected.

May 31. One c.c. injected.

June 2. Sacrificed.

Necropsy.—Findings normal in urinary and gastro-intestinal canal. Gallbladder on external surface hemorrhagic and of an opaque yellowish color. Material removed from it contains large numbers of leukocytes and many gram-negative bacilli. Pus from gallbladder when cultured gave pure culture of gram-negative bacilli—an atypical colon organism similar to the organism originally cultivated from J. P.

Histology.—Section of Kidney: Marked cloudy swelling and vacuolar degeneration. In medulla two-thirds of way to top of pyramid is a small hemorrhage.

Rabbit 5D.—Weight, 12¼ ounces.

May 30. One-tenth c.c. of culture from appendix of Rabbit 1X injected.

May 31. One-half c.c. reinjected.

June 3. Sacrificed.

Necropsy.—On opening the peritoneal cavity the bladder is markedly distended with reddish yellow urine. On folding back, the cecum is adherent to the lower surface of the liver. On peeling back, the stomach, too, is seen to adhere to the lower surface of the liver. These adhesions all seem to be around the gallbladder, extending for some distance along the cecum. The adhesions are of darkish, brownish yellow color, and completely encapsulate the gallbladder, which is covered by the same sort of a fibrinous exudate in its sulcus in the liver. The gallbladder is tense and red where the exudate

has been stripped off. Puncture of the gallbladder shows it to contain a yellowish, thinly purulent material. The smear of contents shows gram-negative bacilli. Kidneys: Slightly enlarged, yellowish-brown, slightly mottled, and on section show a thickening of the cortex. The pyramids and pelves are normal. Cultures from gallbladder and bladder show gram-negative bacilli.

Histology.—Kidney: Intense degeneration of cortex; in some areas, complete necrosis of parenchyma; pelvis and medulla negative.

Rabbit 6D.—Weight, 8½ ounces.

May 30. One-fifth c.c. of appendix of Rabbit 1X injected.

May 31. One-half c.c. reinjected.

June 1. Died.

Necropsy.—Hemorrhagic infarction of descending colon; otherwise negative; culture of kidney shows a gram-negative bacillus. Urine: A few cells; much debris; faintly acid; albumin ++.

Histology.—Descending colon: The entire section is completely infiltrated with red blood corpuscles. The nuclei of the muscles and epithelium are still to be made out. The kidney shows cloudy swelling; otherwise negative.

Rabbit 10D.—Weight, 17½ ounces.

May 30. One-fifth c.c. of culture from appendix of Rabbit 1X injected.

May 31. One c.c. reinjected.

June 1. Died.

Necropsy.—The abdomen is distended. On opening there is an excess of fluid in the peritoneum. The cecum is hemorrhagic and for about 5 inches is covered with fibrinous shreds. The wall is markedly thickened. On opening, the wall is seen to be edematous, the folds hemorrhagic. The remainder of the bowel is injected only where in contact with the cecum. The stomach and duodenum are normal. The kidneys are negative. Cultures from kidney and cecum give gram-negative bacilli. Section 1: Cecum. The mucosa is infiltrated with pus cells and serum. The submucosa is thickened and infiltrated with masses of pus cells.

Rabbit 11D.—Weight, 18 ounces.

May 30. One c.c. of culture of appendix of Rabbit 1X injected.

May 31. One c.c. reinjected.

June 3. Sacrificed.

Necropsy.—The appendix and cecum are normal. The bladder is distended with cloudy urine. The urine contains large numbers of individual cells and masses of cells. On opening the bladder it is seen to be somewhat thickened, hyperemic and has a few hemorrhages. The kidneys are negative externally and on section; the pelvis is normal. Other organs are negative. Culture from bladder, gram-negative bacilli.

Histology.—The kidney shows cloudy swelling. One area has blood on surface of pelvis and no epithelium, probably a postmortem change. The bladder shows marked infiltration with polymorphonuclear leukocytes; hemorrhages and edema.

Rabbit 1E.—Weight, 1 pound 10½ ounces.

June 3. One-half c.c. of gallbladder culture of Rabbit 4D injected.

June 6. Sacrificed.

Necropsy.—Appendix normal; all organs normal. The gallbladder is somewhat thickened and contains thin fluid of a light yellowish color, with numerous white opaque follicles. It is slightly reddened in areas. A smear of the contents shows gram-negative bacilli. Culture shows pure gram-negative bacilli.

Histology.—Kidneys negative, except for marked cloudy swelling. The gallbladder shows areas of necrosis in the wall, with leukocytic infiltration, edema and hemorrhages.

Rabbit 2E.—Weight, 1 pound 12 ounces.

June 3. One c.c. of culture from gallbladder of Rabbit 4D injected.
June 6. Died.

Necropsy.—On opening the peritoneal cavity the upper third of the abdominal organs are seen to be adherent to the parietal peritoneum. The loops of the bowel are injected and glued together and to the liver and stomach. The upper portion of the cecum, in like manner, is glued to the stomach. The liver on its under surface over the region of the bile duct adheres to the stomach and is covered with a brownish-yellow, thick exudate. The omentum is hemorrhagic covering over the stomach. The gallbladder is encased in a sheath of fibrinous exudate which can be peeled back and shows a somewhat distended gallbladder. The adjacent surfaces of the liver also are covered with the exudate. The portion of the stomach adjacent to the liver is injected, and about the pyloris it is considerably thickened and indurated. The inner surface of the stomach shows nothing abnormal. The portion of the bowel which is externally inflamed shows nothing on its internal surface. The cecum and appendix are normal; kidneys normal; heart and lungs normal. There is some stippling of the gallbladder as though of coccidial origin. Cultures made of the gallbladder and intestinal wall show gram-negative bacilli.

Histology.—The heart muscle is negative. There is a large hemorrhage in the kidney at the junction of the cortex and medulla.

Rabbit 4E.—Weight, 1 pound 1¾ ounces.
June 3. One-half c.c. of culture from ulcer of Rabbit 3D injected.
June 5. Died.

Necropsy.—On opening the peritoneal cavity the descending colon and rectum are found thickened, much injected and covered with fibrinous exudate. The cecum at the ileocecal valve shows several areas of minute hemorrhages. Under these areas are seen hemorrhages into one of the folds of the mucous membrane. The stomach and gallbladder are normal; also the kidneys.

Histology.—Cecum: The mucosa is intact. There are marked diffuse and focal hemorrhages into the submucosa and muscularis, but no excess of leukocytes. The edema is marked. Kidney: There is marked cloudy swelling and congestion of the medulla, especially of the pyramidal tips. The pelvis is absolutely negative.

H Series: Rabbit H1.—Weight, 1 pound 15½ ounces.
June 21. One c.c. of culture from urine of N. L. injected.
June 21. Urine alkaline; no cells; albumin, 0.
June 22. Rabbit very ill; diarrhea.
June 23. Died.

Necropsy.—Acute hemorrhagic appendicitis. The appendix shows a few peculiar opaque areas near the top. There are minute hemorrhages in both kidneys and a few depressions on the kidney surface. Bladder culture is negative.

Histology.—Numerous hemorrhages in cortex of the kidney, and blood corpuscles in a few of the collecting tubules. The pelvis is negative.

Rabbit 2H.—Weight, 2 pounds ¼ ounce.
June 21. One c.c. of culture from urine of N. L. injected. Urine acid; crystalline precipitate; no cells; albumin ±.
June 22. Abdomen hard; pain when effort was made to express urine.
June 23. Dead.

Necropsy.—A purulent peritonitis was found. The culture of peritoneum shows a pure gram-negative bacillus. The appendix shows marked proliferation of the epithelial cells and phagocytosis of lymphatic cells; no hemorrhages.

Rabbit H5.—Weight, 1 pound 5½ ounces.
June 22. One c.c. of culture from urine of P. injected. Urinary examination entirely negative.
June 23. Dying. Diarrhea.

Necropsy.—Marked distention with fluid of the intestines, and all along the intestinal canal are hemorrhages. The appendix shows numerous hemorrhages along the course but most marked at its attachment to the cecum. The appendix is filled with fecal material, moist at proximal end, dry at the tip. The mucous membrane is intact. The stomach is normal but there are marked hemorrhages into the duodenum. Culture of bladder gives gram-negative bacilli. Examination of urine negative; no albumin.

Histology.—Duodenum: Hemorrhages into mucosa and submucosa. No inflammatory reaction of leukocytes. The mucous membrane is gone in the hemorrhagic areas, although glands deep down remain. The kidney has cloudy swelling. The pelvis of the kidney is intact, showing no excess of cells.

Rabbit H9.—Weight, 1 pound 1¼ ounces.

June 23. One c.c. of culture from urine of S. injected. Urine alkaline; microscopically negative; no albumin.

June 24. Died. Marked diarrhea.

Necropsy.—The bowel is filled with liquid contents. There are numerous hemorrhages on the appendix, especially toward its base. There are a few hemorrhages into the cecum and numerous hemorrhages into the upper portion of the intestine. There was a hemorrhagic enteritis of the upper portion of the ileum. In this area the ileum is filled with hemorrhagic mucopurulent material. The kidneys and gallbladder are negative. Stomach, duodenum and heart are negative.

Histology.—The ileum shows hemorrhages into the mucosa and inflammatory exudate in the submucosa.

Rabbit H11.—Weight, 1 pound 2½ ounces.

June 24. Injected with bladder culture from Rabbit H8; 1 c.c. given.

June 26. Urine examined; cloudy, acid, microscopically negative; albumin negative.

June 27. Animal almost dead; sacrificed. There is no excess of fluid. In peritoneal cavity the organs are normal. The exterior of the duodenum shows a few hemorrhages. The ileum is distended with fluid and shows injection and scattered small hemorrhages. On opening there are hemorrhages (minute in stomach, larger in duodenum) and throughout a large portion of the ileum. The kidneys are yellowish brown and somewhat fatty. The pelvis is negative. The bladder is negative.

Histology.—The kidney shows cloudy swelling. The appendix and stomach show nothing of note.

K. Series: Rabbit 2K.—Weight, 12½ ounces.

June 28. One c.c. of bladder culture of Rabbit 7G injected. Urine examined before injecting. Microscopically negative; acid; albumin 0.

June 30. Urine acid; single cells and numerous clumps; a few casts; albumin +.

July 3. Urine acid; many cells and groups of cells; sacrificed. Bladder apparently normal; ureters small, apparently normal. The urine expressed from the right ureter negative; from left, yields pus. The kidneys externally are smooth and of a brownish-red color. On section the right kidney is normal. Cortex and medulla are normal and there is no pus in the pelvis, which appears normal. The left kidney is the same as the right, except that there is pus in the pelvis. Bladder cultured; pure gram-negative bacillus found. Smear of pus from left ureter contains pus cells and gram-negative bacilli.

Histology.—Left Kidney: The cortex, except for cloudy swelling, is normal. The medulla shows nothing unusual. The pelvis is filled with masses of pus cells. In parts, especially on the papilla, the mucous membrane is intact. On the surface of pelvis there is a loss of mucosa for a considerable area; in parts small islands of squamous epithelium remain. On one side is seen a large vessel with a thrombus in it. There is a marked infiltration of the mucous membrane with eosinophils. The right kidney is negative; bladder negative.

Rabbit 6K.—Weight, 1 pound 2¼ ounces.

June 28. One and one-half c.c. of culture from bladder of Rabbit 7G injected. Urine examined; microscopically negative; acid; albumin 0.

June 30. Large amount of dark urine; many hyaline and granular casts; precipitate contains no excess of cells; albumin present.

July 3. Sacrificed. Bladder urine contained pus cells. The rabbit seems in excellent condition. On opening the peritoneum, the bladder is practically empty, the stomach and intestines normal. The gallbladder is covered in the lower half with a fibrinous purulent membrane adherent to the liver. The appendix, stomach and duodenum are normal. The bladder contains a small amount of urine, with excess of pus cells, single and in clusters. The urine expressed from the cut ureter on the right side yields pure pus; on the left side very few epithelial cells. A smear of the pus from right ureter contains gram-negative bacilli. A smear of urine from bladder shows a gram-negative bacillus in pure culture. The right kidney is of a brownish yellow color and smooth on section. The pelvis of the kidney exudes thick yellow pus. The cortex is opaque and yellow; the medulla shows nothing unusual. The left kidney on section shows no pus in the medulla, but in every other way resembles the right. The ureter of the right kidney is considerably smaller. Cultures of gallbladder contents shows a gram-negative bacillus.

Histology.—The epithelium on the pyramid is intact; over the opposite side of the pelvis the epithelium is lost and the surface is made up of a mass of pus cells. There is considerable infiltration of the lining of the pelvis. In the left kidney there is no pus in the pelvis, but a slight increase in connective tissue in the cortex. The bladder is negative.

Rabbit 7K.—Weight, 1 pound 11¾ ounces.

June 28. One and one-half c.c. of bladder culture of Rabbit 7G injected; Urine examined; a few cells and no albumin were found.

June 30. Died.

Necropsy made immediately after death. Peritonitis present; excess fluid; coccidiosis, especially about stomach and duodenum. Bladder contains a small amount of normal urine. Left kidney shows two small yellow opaque areas in cortex. Otherwise negative except for the injection of a portion of cecum near valve. Culture of bladder heavy growth in broth of gram-negative bacillus.

Histology.—There are minute abscesses in kidney, both in the cortex and the medulla.

Rabbit 15K.—Weight, 1 pound 4½ ounces.

July 4. One c.c. of bladder culture from Rabbit 2K injected. Urine examined; microscopically negative, acid; albumin 0.

July 5. Diarrhea; many pus cells and epithelial cells; acid, albumin + +.

July 6. Diarrhea; many pus cells and groups of cells; acid; albumin + +.

July 7. (Weight 15½ ounces; ill); urine loaded with pus cells and groups of cells; acid; albumin + +.

Died between July 7 and 8.

Necropsy.—On opening the peritoneum, hemorrhages are found in the lower portion of the retrovesical tissue; bladder distended with large amount of urine, which does not contain excess of pus cells. Bladder wall on top shows slight thickening; kidneys normal. Stomach and duodenum normal. Gallbladder adhered to the liver and covered with omentum, which is matted together and can be separated from the gallbladder in a thickly purulent area. Along the line of cleavage there is a purulent membrane; appendix, stomach and duodenum normal. Heart negative. Kidneys smooth, uniform in color, on section show nothing unusual. Coccidiosis of liver and gallbladder. Vagina much reddened, and in an area about 3 mm. across there is an opaque, yellowish membrane.

Histology.—The kidneys show nothing abnormal. The vagina shows a pseudomembranous inflammation of the wall, with considerable necrosis.

Rabbit 17K.—Weight 1 pound 8¼ ounces.

July 4. One c.c. of bladder culture of Rabbit 2K injected. Urine examined; microscopically negative; acid; albumin 0.

July 5. Diarrhea. About 20 c.c. of urine expressed; contains a few cells and groups of cells, probably no excess; acid; albumin +.

July 6. Diarrhea; no excess of cells; acid; albumin 0.

July 7. Weight, 1 pound 3½ ounces. Urine examined, negative; cloudy with amorphous precipitate; acid; no albumin.

July 8. Died.

Necropsy.—On opening there is an excess of fluid in the peritoneum. The bladder is markedly distended; the intestinal canal is apparently normal; kidneys are smooth, of a uniform color on the surface. The gallbladder is studded with white opaque areas, and in addition numerous small hemorrhagic areas. The gallbladder is smooth on the external surface. The kidney on section shows nothing unusual. The pelvis is smooth and contains no pus. Nothing is obtained from the ureters.

Histology.—The kidneys show nothing of note.

Series L: Rabbit L1.—Weight, 2 pounds.

July 12. Three c.c. of a sodium chlorid suspension of bacilli from the urine of C. injected. Suspension approximately the same as in the urine. Urine examined; a few cells; urine clear, alkaline; acid to phenolphthalein; no albumin.

July 13. No excess of cells; acid; albumin 0.

July 14. A few cells; acid; albumin +.

July 15. Large number of pus cells; acid; slight amount of albumin. Sacrificed.

Necropsy.—Bladder urine negative; no pus obtained from either ureter; kidneys are negative; no vaginitis. The gallbladder is hemorrhagic, infected and full of pus. The findings otherwise are entirely negative.

Histology.—The kidneys are entirely negative.

Rabbit 3L.—Weight, 2 pounds 1¼ ounces.

July 13. One-half c.c. urine culture of C. injected. Urine examined; microscopically negative; alkaline; albumin 0; cloudy, amorphous precipitate.

July 14. Urine examined; excess of pus cells; acid; albumin 0.

July 14, p. m. One-half c.c. reinjected.

July 15. Urine examined; excess of pus cells; acid; albumin +; weight, 1 pound 11¼ ounces.

July 15 to 17. Died.

Necropsy.—Much decomposed. Bladder urine filled with cellular, apparently epithelial, debris. Epithelial debris from both ureters. Gallbladder shows marked injection, and suppuration; portion of stomach at pyloric end adhering to it is hemorrhagic. Kidneys are large and bluish-brown in color. On section there are seen numerous yellow dots and lines in the medulla; marked degeneration of the cortex; numerous linear abscesses, extending to papilla in the medulla; intense congestion.

Histology.—There are innumerable abscesses in medulla.

Rabbit 4L.—Weight 2 pounds.

July 13, 3:30. One-quarter c.c. of urine culture of C. injected. Urine examined; microscopically negative; alkaline; albumin 0; cloudy, amorphous precipitate.

July 14. Microscopically negative; acid; albumin +.

July 14 p. m. One-quarter c.c. reinjected.

July 14 to 15. Died.

Necropsy.—On opening the peritoneum the descending colon for a distance of about 4 inches is of dark, reddish-brown color and considerably distended. There is a sharp transition at both ends to practically normal intestinal wall. On opening up the colon this region is seen to be markedly hemorrhagic and

somewhat thickened showing the same abrupt transition to normal tissue. The kidneys show a few small hemorrhages in the surfaces. The pelvis shows nothing abnormal.

Histology.—The kidneys are entirely negative.

Rabbit 2F.—Weight, 1 pound 15¾ ounces.

June 9. One c.c. of urinary culture of B. injected. Urine negative for albumin and pus cells.

June 10. Urine acid, cloudy with amorphous precipitate; no cells; albumin +.

June 12. Acid, cloudy, precipitate largely amorphous; some pus cells; many bacteria; albumin +.

June 14. Weight, 1 pound 12¼ ounces. Urine thick and stringy, full of masses and strings of amorphous material; acid; no cells; few bacteria; albumin ±.

June 15. Urine very cloudy; full of amorphous material; a few pus cells; albumin +.

June 16. Urine cloudy; much amorphous material; some pus cells; albumin ±.

June 17. Sacrificed.

Necropsy.—Findings were entirely negative; examination of bladder urine showed many pus cells as well as some amorphous material; urinary culture showed a gram-negative bacillus and gram-positive coccus.

Histology.—Kidney showed slight infiltration of a portion of the pelvis; marked cloudy swelling of parenchyma.

Rabbit 3F.—Weight, 1 pound 6 ounces.

June 9. One-half c.c. of urinary culture of B. injected. Urine negative for albumin and pus.

June 10. Urine slightly acid; brownish-yellow; cloudy with amorphous precipitate; few bacteria; no cells; albumin ++.

June 12. Acid urine; cloudy, amorphous precipitate and masses of shreds and debris of all kinds; albumin ++.

June 14. Weight, 1 pound 1¼ ounces. Urine very cloudy; full of pus cells and motile bacteria; albumin ++; acid.

June 15. Urine cloudy; full of pus cells; albumin ++.

June 16. Urine cloudy; full of pus cells; albumin +. Sacrificed.

Necropsy.—On opening the peritoneal cavity the serous surfaces are everywhere smooth. The bladder is distended with cloudy urine, the ureters are not distended; the kidneys are smooth on the surface but in two places on left and in one place on the right there is an opaque, yellowish area from 1 to 3 mm. in diameter. On section through areas they are seen to extend into the medulla in a long, yellowish streak practically to the papilla. The pelvis shows nothing unusual. The gallbladder is distended; outer surfaces are smooth, and contents consist of thick purulent material. Some coccidiosis of liver. The stomach, duodenum, appendix and cecum show nothing unusual. The pericardium adheres over the entire upper portion of the heart and is covered with a thick, creamy exudate. Culture from the bladder shows a gram-positive coccus and a gram-negative bacillus; also from the pelvis of the kidney and the gallbladder a gram-positive coccus and a gram-negative bacillus; from an abscess of the kidney a gram-positive coccus alone; from the heart, a gram-positive coccus alone.

Rabbit 4F.—Weight, 1 pound 4¾ ounces.

June 9. One c.c. of culture injected. Urine negative for albumin and pus.

June 10. Urine acid; pretty clear; slight amorphous precipitate, no cells or bacteria; albumin +.

June 12. Urine acid; cloudy, amorphous precipitate; no cells; few bacteria; albumin +.

June 14. Weight, 1 pound 3¾ ounces; urine very cloudy; stringy, amorphous masses; no cells; acid; albumin ±.

June 15. Urine cloudy; stringy substance present; few cells; acid; albumin +.

June 17. Sacrificed.

Necropsy.—Findings are entirely negative. Urine cloudy, with amorphous precipitate; no cells. Urine culture showed a gram-positive coccus and a gram-negative bacillus.

Histology.—Kidneys contain numerous pus cells in groups in the pelvis and in the lumen of the ureter. The bladder is negative.

Rabbit 3G.—Weight, 1 pound.

June 17. One c.c. of suspension of culture from kidney abscess of Rabbit 3F injected intravenously.

June 19. Found dead; ill the afternoon of June 18.

Necropsy.—On opening the peritoneum a portion of the ileum just above the ileocecal valve is found reddened and hemorrhagic. The most marked area is found about 1 foot above the ileocecal valve. This portion of the bowel on opening is studded with small hemorrhages, and on a portion of the wall there is a diphtheritic exudate. The appendix is thickened, and in an area about 1 inch below its attachment to the cecum is deeply injected and hemorrhagic and of an opaque, yellowish color. The appendix on opening shows in this hemorrhagic area a hemorrhagic exudate and considerable necrosis of the wall. The urine is cloudy, and on microscopic examination shows some casts and a few pus cells, and a considerable number of epithelial cells. The kidneys are much enlarged, opaque, of yellowish color, studded with groups of minute hemorrhages which give the kidney a mottled appearance. On section the hemorrhages are seen to be limited to the cortex and resemble the appearances on the outside. The heart is negative. A pure culture of a gram-negative bacillus was obtained from the submucosa of the appendix and from the bladder. The kidney cortex culture was negative.

Histology.—The kidney shows numerous hemorrhages in the cortex and considerable degeneration and necrosis of tubules. There are also some minute areas of infiltration in the cortex.

Rabbit 4G.—Weight, 1 pound 8¾ ounces.

June 19. One c.c. of culture from abscess of left kidney injected intravenously. Urine acid; negative.

June 20. A few cells; some amorphous material; slightly acid; no albumin.

June 21. Sacrificed.

Necropsy.—Peritoneum clear; appendix and cecum negative. Gallbladder hemorrhagic. The stomach has few small hemorrhages in the cardiac end; a small ulcer (?). The duodenum has six to eight hemorrhages just beyond the pyloric ring. The heart is negative. The kidneys are large, yellow and pale; cortex smooth but studded with minute hemorrhages over the entire surface; same in both kidneys. There are also small areas of more opaque, yellow appearance. The bladder shows numerous small submucous hemorrhages, one of somewhat larger size. Urine from the bladder shows albumin, epithelial cells, casts and leukocytes. Cultures of the urine are negative.

Histology.—The kidney cortex has no hemorrhages in the sections studied. There is marked degeneration and necrosis of epithelium in areas. The stomach and duodenum show hemorrhages in the mucosa.

Rabbit 7G.—June 20. One c.c. from abscess of left kidney, Rabbit 3F injected. Urine negative; acid.

June 21. Urine contains a few pus cells; acid; albumin +.

June 22. Urine acid; numerous pus cells; albumin +; diarrhea.

June 23. Urine acid; numerous pus cells, albumin +; diarrhea.

June 25. Diarrhea; urine contains numerous cells and groups of cells; albumin +.

June 27. Dying; chloroformed.

Necropsy.—On opening the abdomen the bladder is markedly distended with urine. The serous surfaces are everywhere smooth. The only unusual thing is a peculiar yellowish discoloration of that part of the ileum just above the ileocecal valve. The gallbladder is distended and filled with a thick yellowish material. On opening the chest the right ventricle of the heart is seen to be much dilated and of a yellowish color. The lungs are negative. The bladder shows a perfectly normal mucous membrane. There are no hemorrhages of the wall. The appendix is normal; the cecum is somewhat thickened near the ileocecal valve, and for a distance of about 6 inches above is somewhat thickened and in patches covered with a yellowish membrane firmly adhering to the mucous membrane. The stomach and doudenum show nothing unusual. The right kidney is of a brownish-red color and shows one or two lighter areas. On section it is of about normal thickness and the same color as the external surface. The medulla is somewhat edematous. There is pus in the pelvis of the kidney, but no hyperemia. The ureters and bladder are normal. On the left kidney are opaque yellowish areas which are limited to the cortex. The pelvis contains pus; otherwise nothing unusual. Cultures from the gallbladder and bladder contain a pure gram-negative bacillus.

Histology.—There is infiltration about many glomeruli. The pelvis of both kidneys shows an intact mucous membrane, but just below the epithelium there is a marked infiltration with leukocytes; also in the tips of the papillae. In the colon a diphtheritic membrane covers a large area of the surface. There is complete destruction of the mucosa and submucosa, and massive infiltration and fibrosis of the entire wall.

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EXPERIMENTAL PYELITIS IN THE RABBIT *

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Numerous attempts have been made in the past to produce pyelitis by injecting bacteria into the bladder, with the idea of getting an ascending infection, and by injecting organisms into the circulation and expecting them to set up lesions in the kidney during the process of being filtered out. It has long been known, and it is becoming increasingly more evident, that bacteria during any infection, if they reach the blood stream, are likely to be excreted in the urine. This applies to acute as well as chronic diseases. The kidney acts as a filter to remove bacteria from the circulation. If this is constantly taking place, we must naturally assume that the kidney has a great resistance to these organisms or that the bacteria are carried out so rapidly that they do not have an opportunity to multiply.

The two possibilities for infection are: (1) a loss of resistance of the kidney or an increase in the virulence of the organism; and (2) a stopping up of the natural washing-out of the urinary passages. The latter one of these factors has been studied by a great many observers, who have all showed that anything that obstructs the flow of urine makes possible an infection of the kidney, whether the infecting organism is introduced from the bladder or by the blood stream. Furthermore, the work of Stewart[1] on ascending infection of the kidney by ureteral trans-

* From the O. S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital.
* Read before the Section on Diseases of Children at the Sixty-Eighth Annual Session of the American Medical Association, New York, June, 1917.

1. Stewart: A Study of Ascending Infection, etc., Univ. Pennsylvania Med. Bull., 1910, **23**, 233.

plantation into the rectum has shown that the infection spreads upward by the lymph channels, and that the spread by the lumen of the ureter is less of a factor than formerly supposed. In this work, however, the lymph channels were cut and naturally offered excellent opportunity for the spread of infection. Hess[2]

Fig. 1.—Spontaneous pyelitis (distended ureters and pelvis).

came to the conclusion that something more than the simple injection of colon bacilli into the bladder was necessary to produce ascending infection in the rabbit. The previous injection of turpentine or other irritant

2. Hess: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1913, **26**, 135.

into the bladder was sufficient to bring about an involvement of the upper urinary tract. More recently Eisendrath and Schultz[3] have attempted to show that infection of the bladder with *Bacillus coli,* streptococci and *B. proteus* travels up the periureteral lymphatics. They used rabbits and dogs in their work. They described very extensive changes in nineteen of thirty-eight animals but recovered organisms from the kidney in only six of their positive cases in their first series and in none of their second. It seems that in view of the negative bacteriologic results, the casual relationship of the lesions and the organisms injected must be left open.

Fig. 2.—Hematogenous cortical abscesses.

It is not our object here to go into a discussion of the etiology of pyelitis, but merely to point out that with an unobstructed urinary tract there have been practically no constantly positive results in the production of pyelitis experimentally, either by intravenous or intracystic injection. In a previous series[4] of intravenous experiments we were able to produce kidney lesions in only eight out of sixty-six animals injected with colon bacilli, and in four out of eleven animals when injected with a mixture of colon bacilli and pneumococci, the tendency of the organisms to

3. Eisendrath and Schultz: Jour. Med. Research, 1917, **35**, 295.
4. Helmholz, H. F., and Beeler, Carol: Focal Lesions Produced in the Rabbit by Colon Bacilli Isolated from Pyelocystitis Cases, Am. Jour. Dis. Child., July, 1917.

SUMMARY OF INJECTIONS

Series	No.	Kidney Lesions				Other Organs Involved
		Total	Pyelitis	Abs.	Bact.	
A. Intravenous Injection						
I	10	6	4	4	..	Hemorrhage into lung.
II	3	2	2	1	..	Abscess of seminal vesicle.
III	4	2	3	1	..	Hemorrhage of rectum and rectum.
IV	2	2	1	2	..	0
V	5	4	2	3	..	0
VI	3	2	1	2	..	Chronic nephritis.
VII	3	1	1	0	..	Generalized peritonitis.
VIII	3	1	1	1	..	0
IX	3	2	2	2	..	0
	32	22	16	16		
B. Intracystic Injection						
II	2	0	0	0	..	Infected Right Testis.
IV	2	2	2	0	..	0
V	5	3	1	0	2	0
VI	2	2	2	1	..	0
VII	2	2	2	0	..	0
VIII	2	2	2	0	..	0
	15	11	9	1	2	
C. Rectal Injection						
VII	2	0	0	0	..	0
VIII	2	0	0	0	..	Gram-negative bacillus.*
D. Oral Administration						
V	5	0	0	0	..	1x Bladder Gram-positive coccus; Gram-negative bacillus.
VI	4	0	0	0	..	2x Bladder Gram-negative bacillus.

* From bladder urine.

produce lesions in other organs being just as great as in the kidney. During the course of the experiments just cited, in controlling the urine of all animals before injection, we found a female rabbit with a constantly purulent urine. Cultures taken from a catheterized specimen of urine showed a pure culture of a gram-negative bacillus, that had the following characteristics:

The organism grew well on all mediums. There was a profuse yellowish growth on agar. Gelatin was not liquefied. Litmus milk was rendered acid and coagulated; but there was no digestion of the casein. Indol was produced. There was acid and gas formation in dextrose, lactose and saccharose. The organism thus belongs in the group of *Bacillus coli-communior.*

HISTORY

Feb. 17, 1917. The first urine examined showed about 150 pus cells to a low power field.

February 9. There was a large excess of pus cells.

February 10 to February 20. Daily examination revealed a large number of pus cells, single and in groups.

February 20. A catheterized specimen of urine was cultured in litmus lactose agar, plain agar and broth.

February 21. There are innumerable colonies on both plates.

February 23. Specimens were expressed every other day showing albumin and casts in addition to pus cells.

March 3. Another catheterized specimen was cultured; pure culture same as obtained on February 20.

The animal was chloroformed, as it was getting very much weaker.

Necropsy.—On opening the peritoneal cavity the abdominal organs all appear practically normal. The bladder is distended with urine. The stomach, gastro-intestinal canal, spleen, liver, gallbladder, heart and lungs show nothing abnormal. On dissecting free the overlying organs the ureters on both sides are seen to be considerably dilated, as far down as the brim of the pelvis. Beyond this point they appear to be of normal diameter. No apparent pathologic constriction in the ureter at the brim of the pelvis was visible. The pelves of both kidneys were smooth, and of a reddish brown color; the capsule stripped readily. On section the cortex was of normal thickness, the striations regular. The outer portion of the medulla was deeply congested. The pelves in both kidneys were markedly distended, as seen in Figure 1. The left kidney pelvis was filled with thin purulent material, the right practically empty. The bladder urine showed only relatively few leuko-

cytes, numerous casts and much débris. Cultures taken from the bladder and right kidney pelvis yield a pure culture of gram-negative bacillus.

Histology.—The bladder mucous membrane in the entire section is practically intact. In areas there are small clusters of lymphocytes; there are practically no polymorphonuclear leukocytes to be seen in the section. Kidney: There is considerable degeneration of the epithelium and dilation of collecting

Fig. 3.—Ascending pyelitis (distended ureter).

tubules; in areas focally there is considerable increase in connective tissue. In the medulla, especially toward the tip of the papilla, there is marked leukocytic infiltration about the tubules. Eosinophil cells are much in evidence. The mucosa of the pelvis is apparently thickened and shows considerable infiltration with leukocytes. The pelvis is filled with masses of polymorphonuclear leukocytes.

The colon bacillus isolated from the catheterized specimen of urine was used in nine series of experiments. The technic used in the experiments was as follows: The intravenous injections were made with a bacterial suspension that was made by centrifuging 15 c.c. of a twenty-four hour broth culture and suspending the bacilli in 5 c.c. of physiologic sodium chlorid solution. One c.c. of this suspension was injected into the ear vein. The intracystic injections were made after carefully catheterizing the animals and then allowing 5 c.c. of the

Fig. 4.—Ascending pyelitis (cortical abscesses).

bacterial suspension to run into the bladder.[5] In the feeding experiments 8 c.c. were injected. In the first series of ten animals which were injected intravenously, six showed lesions of the kidney; in four instances a pyelitis, and in four

5. Three control animals injected with a dilute solution of gentian violet showed that no fluid passed beyond the bladder.

instances cortical or medullary abscesses. In two instances, as is evident from the table, both were present. In Series 3 and 9 also, all the injections were intravenous. In the other series the same culture was used in the injections given intravenously, intracystic and either rectally or orally, in order that the results could be more accurately compared.

In the thirty-two animals of nine series injected intravenously, twenty-two showed lesions of the kidney, in sixteen instances pyelitis and in sixteen instances cortical (Fig. 11) or medullary abscesses. In ten instances there were both abscesses and pyelitis. No doubt if the animals with abscesses had lived they would all have developed a pyelitis also. Of the thirty-two animals only three showed lesions outside of the urinary tract that could be ascribed to the colon bacillus: one instance each of abscess of the seminal vesicle, hemorrhage of the cecum, and generalized peritonitis. The peritonitis was due in large measure no doubt to extensive coccidiosis. The absence of lesions in other organs is just as striking as the localization of the organism in the kidney in twenty-two of the thirty-two cases.

The intracystic injections consist of six series, totaling fifteen animals. In four series of two animals each, pyelitis was produced in 100 per cent. In the fifth only one had a definite pyelitis, but in two others gram-negative bacilli were isolated from the pelvis of the kidney. In the sixth series neither one of the two animals injected acquired a kidney infection. Figure 3 shows the swelling of the ureter, Figure 4 the one kidney with cortical lesions in the intracystic series.

Four animals that received rectal injection and nine that were repeatedly fed the colon culture showed no lesions of the urinary tract, except that in one instance in the rectal series and in three instances in the oral series, colon bacilli were isolated from the bladder but not from the kidney.

The serum of a rabbit immunized to the specific organism isolated from the urine agglutinated the organism in a dilution of 1:2,000. It did not agglutinate several strains of *B. coli-communior* isolated from human cases of pyelitis nor several strains of *B. coli* isolated from rabbit feces. A *B. coli-communior* isolated from the feces of the original pyelitis rabbit was agglutinated in the same dilution as the colon bacillus isolated from the urine.

COMMENT

The remarkable specificity of this organism is of interest from two very different points of view: first, it enables us to study better than ever before the etiology and probably the treatment of kidney infections, and secondly, from the purely biologic point of view, it shows us the extreme specificity the colon bacillus can acquire in localizing in the kidney, on intravenous injection.

These experiments prove without a doubt that pyelitis can be produced experimentally by intravenous injection as well as by injection into the bladder. What the route of the infection is from the bladder to the kidney we must leave an open question at this time. One point that we do wish to make, however, is that this work shows that if the organism has a specific affinity for the kidney its introduction into the bladder can be of very serious consequence to the individual. It will take further study to determine whether the bacilli reach the kidney via the lumen, the lymphatics or the blood stream. Experiments in that connection are at present under way.

If we compare these experiments with the results obtained by the injection of organisms isolated from human pyelitis cases, the specificity of this organism is even more striking. In sixty-six animals injected with different strains of *B. coli* only eight showed kidney lesions, and twenty-one lesions of the other organs; with this organism, of thirty-two animals injected twenty-two showed kidney lesions and in only three instances were the other organs affected.

The so-called epidemics of pyelitis that have been described from various European clinics might well be ascribed to an organism that, as this organism, has a specific affinity for the kidney tissue.

The specific localization of organism in tissues has been of great interest, especially since the work of Rosenow on the pneumostreptococcus group. Rosenow[6] in his work on the colon bacillus and streptococcus in gastro-intestinal infection, especially of the appendix, showed that the combination had specific tendencies to localize but that the colon bacillus alone had no such powers. Our work on the colon bacilli of human pyelitis cases led to practically the same result. With this organism the specificity manifests itself not only in that the kidney is involved in about 70 per cent. of the injections, but that in less than 10 per cent. of the injections is there involvement of any other organ. The intracystic infections are not of much importance in this connection because the distribution of the bacteria is not so widespread throughout the system as with the intravenous injections.

6. Rosenow: Jour. Infect. Dis., 1915, **16**, 240.

SUMMARY

1. Infections of the kidney were produced in 70 per cent. of all animals injected by the intravenous or intracystic route with a colon bacillus obtained from a rabbit with spontaneous pyelitis.

2. The organism, a *Bacillus coli-communior*, had a specific tendency to localize in the kidney tissues.

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THE EFFECT OF CHILLING ON EXPERIMENTAL PYELONEPHRITIS IN THE RABBIT*

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In a recent publication Helmholz and Beeler[1] showed that colon bacilli isolated from cases of pyelocystitis produced focal lesions in the kidney of the rabbit in only a relatively low percentage of cases. In only 8 of 66 animals injected did the colon bacilli show any selective tendency to localize in the kidney. The tendency to produce kidney lesions was more evident when a mixture of colon bacilli and streptococci were injected. This second series of animals was too small, however, to draw any very definite conclusions. It seemed of interest to determine whether there were other factors that might influence the localization of the colon bacilli in the kidney. The first factor to suggest itself was chilling, because of the frequency with which nephritis develops after chilling and because of the fact that pyelitis occasionally follows chilling.

In the earlier experiments the animals were chilled immediately after intravenous injection, in the later from 3 to 4 hours after injection, because it was considered that after the bacteria had been scattered around in the different organs the effect of the chilling would make itself more manifest. In the first series of experiments several strains of colon bacilli were used. In the second series of animals a streptococcus isolated from a human case of pyelitis was used. Twenty experiments were carried on with colon bacilli and 9 with streptococci.

The technique employed was the same that as used by Helmholz and Beeler.[1] For 24 hours before injection the animals were put on bread and water diet to render the urine acid. In every instance the urine was examined before injection for pus cells and albumin. The animals were injected into the ear vein with ½ to 1 c.c. of a bacterial suspension of broth cultures. At intervals varying from a few minutes to 4 hours after the injection the animals were thoroughly wetted and exposed in a cold room before

* From the Otho S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital, Chicago.

an electric fan for periods varying from 10 minutes to an hour. In order to control the effect of the chilling 2 animals in each series were chilled without a previous intravenous injection. None of these animals showed any effects in the urinary tract from the chilling, nor, so far as we could tell, did they suffer in any way. The colon bacilli used in these injections were all recently isolated from acute cases of pyelitis. The streptococci were isolated from a case of pyelitis that was of some standing.

The results of the experiments with colon bacilli have been tabulated in Chart 1. Of the 20 animals injected, 4 should be excluded, because they all died within the first 24 hours. Of the remaining 16, 8 showed at one time or other a considerable amount of pus in the urine. Of these, R M 8 showed a large number of pus cells for a period of about 3 days, they gradually decreased so that in several days the urine contained no pus. Then for a period of about a week there was a large number of pus cells, both single and in groups, and this condition prevailed until the time of death. The autopsy and microscopic examination showed no evidences of pathologic change in the kidney.

The second case with pus in the urine showed at autopsy streaks that resembled medullary abscesses but on histologic examination these were seen to be due to chronic nephritis. In a third case, R 22, the pus in the urine was found to have been an admixture from an acute endometritis. A fourth animal, R 26, that showed numerous pus cells in the urine following the injection, lived two weeks longer than any of the other animals that showed abscesses of the more acute type. At autopsy there were no pus cells in the urine and the kidney lesions were of a subacute or even chronic type. The character of the lesions made it practically impossible to decide whether they were the result of the injection or not.

Of the other 5 animals that showed pyuria, 4 had medullary abscesses, 1 cortical abscesses, and 1 definite evidence of a pyelitis. The last animal was the same that showed the cortical abscesses. Of the 3 animals that showed pus in the urine without definite kidney lesions histologically, 1 with acute endometritis must be definitely excluded. The other 2, especially R M 8, referred to above, might have shown some kidney lesions if more extensive histologic study of the kidney had been made. We make this statement because in other instances where the pelvis has been found distended with pus at autopsy, it has been difficult to

demonstrate histologic changes in the kidney tissue in the sections studied, contrary to all expectations. Experiments R 21 and R 23 are of special interest because of the extensive hemorrhages that were seen in the medulla. Small hemorrhages were seen on the surface of both kidneys and on the cortex. The portion adjoining the cortex was a typical burgundy red color, that ran down in small streaks toward the papilla. In experiment 23 with these hemorrhagic areas, there was necrosis of the epithelium with leukocytic infiltration, surrounding large masses of bacteria. In the kidney of R 21 no such areas of infiltration were seen. Both of these animals lived over 24 hours, but less than 48 hours. Five of the animals injected showed a cholecystitis of varying degrees of intensity, 1 an appendicitis, 2 pneumonia and 1 an acute edometritis.

It will thus be seen from the figures that 4 and possibly 5 (R 21) of the 16 animals, that lived at least 24 hours, showed definite inflammatory changes in the kidney. R 21 showed very extensive hemorrhages of the medulla without, however, any inflammatory changes. Thus in about 25% of the animals injected, kidney lesions were produced.

At the time that we carried on these experiments it did not occur to us to make a sufficient number of control injections, without chilling. At a later date it was impossible to do this because fresh cultures were no longer available from these same cases of pyelitis. However, the large series of cases reported by Helmholz and Beeler[1] can readily serve as control experiments for this series.

This series of 20 experiments tends to show that chilling has a definite effect in producing kidney infection.

The results of the experiments with intravenous streptococci injection followed by chilling have been tabulated in Chart 2. Two animals, R 17.9 and R 17.10, were injected with the streptococci and were not chilled. The first animal showed a negative urine until about 2 weeks after injection when a small number of pus cells were found in the low power field. The autopsy was done about a week later and showed negative findings of the kidney and urinary tract. On microscopic examination, however, there were numerous small abscesses in the medulla. The pelvis showed no inflammatory change. The second control, R 17.10, showed on one day a few pus cells and at no other time during the month following the day of injection were there any pus cells

in the urine. At autopsy, too, the kidney showed nothing abnormal. On microscopic examination, however, there were seen numerous longitudinal abscesses in the medulla and an excess of eosinophile cells. In this connection, it is of interest to note that the changes found in the medulla in these 2 cases resemble the changes that one usually sees in positive cases during the first 4 or 5 days following an injection. If there had been any lesions in other organs at autopsy it might be supposed that the organisms localized elsewhere and secondarily involved the kidney; but in neither instance was this the case. It is rather difficult, therefore, to explain the acute nature of the infiltration in view of the fact that it was almost a month after the injection in each case that the animals were sacrificed.

Of the 7 animals that were injected with streptococci and later chilled, all showed pus cells in the urine at one time or another. Of the 7 animals, 2 showed medullary abscesses, and 2 a pyelitis, unilateral in the one instance and bilateral in the other. None of the 3 remaining animals presented any lesions on histologic examination. This is the more remarkable because 2 of them showed a large number of pus cells in the urine. Taking up the animals that showed pus in the urine but no pathologic findings, it is barely possible that the condition was practically healed before the time of post mortem examination. In animal R 17.12 3 days after the injection and chilling, the urine was loaded with pus cells, single and in groups, and the smear showed a large number of streptococci. Then for a period of about 2 weeks the urine constantly contained pus cells and a few casts. For a short period the pus decreased, to increase again and remain constant up to the time of death. At autopsy the bladder was filled with urine containing only a relatively small number of pus cells. There was nothing in the genital tract to explain the large amount of pus and there was nothing in the histologic examination of the urinary tract to account for the pus.

R 17.8 showed a large number of pus cells and casts beginning 2 days after injection until the time of death. At autopsy the vagina and urethra were entirely negative. The bladder, however, showed several small hemorrhages. The kidneys and ureters, on the other hand, showed no inflammatory infiltration.

R 17.4, the third of these cases, showed pus in the urine beginning about 10 days after the injection, then for a period of about 2 weeks it showed a considerable number of pus cells

which later disappeared. On histologic examination the only thing found was an increased number of eosinophiles in the glomeruli.

Thus of the 7 animals injected, 4 showed definite kidney lesions, 2 of the medulla, 2 of the pelvis. The other 3 showed pyuria for varying periods of time. No definite cause could be found for this pyuria at autopsy in either the urinary or genital tract. As indicated in the chart in one of these animals there were extensive hemorrhages into the muscles of the thigh, a second had pericarditis and a third endometritis.

The fact that the 2 control animals also showed lesions makes it rather difficult to say how much effect the chilling had in producing kidney lesions in this series.

In all the work our efforts have been directed toward producing pyelonephritis with some degree of constancy. This is absolutely essential for a study of the development and treatment of this condition. We have recently found a colon bacillus[2] that localized in the kidney tissues of the rabbit quite constantly; therefore, we did not feel justified in extending these experiments any further. This strain of colon bacillus was isolated from a spontaneous pyelitis of the rabbit and the incidence of kidney takes was almost 70%. This observation is naturally of the utmost significance. It seemed of interest, however, to call attention to the definite tendency of a streptococcus isolated from a human pyelitis to produce kidney lesions. This tendency was very much more marked than in any colon organism that we have so far isolated from a human case of pyelitis. This tendency of the streptococcus to produce kidney lesions also is of great interest. In this series, as in a previous one, we found that one of the cases of pyelitis was unilateral, showing that conditions may be sufficiently different in the two kidneys to produce lesions in one and leave the other one entirely unharmed.

Summary.—In a series of 20 intravenous injections of colon bacilli followed by chilling, about 25% showed focal lesions in the kidney, as compared with 12% of those receiving merely intravenous injection. (Helmholz and Beeler.)

Secondly, a streptococcus isolated from a case of pyelocystitis showed in 66% of the animals injected focal lesions in the kidney.

1.—Helmholz and Beeler: Focal Lesions Produced in the Rabbit by Colon Bacilli Isolated from Pyelocystitis Cases, Am. Jour. of Dis. of Child., Vol. XIV., 1917, 5-24.

2.—Helmholz and Beeler: Experimental Pyelitis in the Rabbit, Jour. Am. Med. Ass'n. Vol. LXIX., 1917, p. 898.

CHART I. DATA OF EXPERIMENTS.

SERIES I.

ANIMAL	DATE OF INJECT.	DATE OF DEATH	TIME CHILLED	URINE PUS	KIDNEY	MICROSCOPIC	REMARKS
RM8	12/7/16	12/29	15 Min.	12/12 ### 12/14 0	0	6 0	Marked Pyuria later cleared up.
RM9	12/13	1/8	10 Min.	#	Cortical Streaks	Chronic Nephritis.	
RM10	12/12	12/17	10 Min.	0	0		Purulent Cholecystitis.
RM11	12/13	1/3	10 Min.	12/29	0	0	Catarrhal Cholecystitis.
RM12	12/22	12/23	10 Min.	0	0	0	Died inside 24-hr. Period.
RM13	12/22	1/8	10 Min.	12/29# 1/8#	Hemorr. Absc.	Medullary Absc.	
RM14	12/22	12/29	15 Min.	0	0	0	Cholecystitis.
RM15	12/22	1/5	15 Min.	0	0	0	Fractured Femur.
R 8	12/23	12/28	1 Hr.	###	Pyelitis Absc.	Cort. Absc. Pyelitis	Cholecystitis.
R 9	12/23	12/24	1 Hr.	0	0	0	Died within 24-hr. Period.
R 10	12/23	12/26	1 Hr.	?	Absc.?	No Absc.	Cholecystitis.
R 18	12/23	12/23					Died within few hrs.
R 20	12/29	12/29	1 Hr.	0	0	0	Died within 4½ hrs.
R 21	12/29	12/30	½ Hr.			No Absc.	Appendicitis.
R 22	12/29	1/3	½ Hr.	##	0		Acute Metritis.
R 23	12/29	12/30	½ Hr.	#	Hemorr.	Abscesses.	
R 24	12/29	1/18	1 Hr.	0	0		Stom. Ulcer.
R 25	12/31	1/18	1 Hr.	0	0	0	
R 26	12/31	1/18	1 Hr.	##	0	Chronic Nephritis	Pneumonia.
R 27	12/31	1/1	1 Hr.	0	Hemorr.		
R 28	12/31	1/5	1 Hr.	#	0	Medullary Absc.	Pneumonia. Pericarditis.

SERIES II.

R17.1	1/25	2/6	1 hr.	##	Unilat. Pyelitis	Unilat. Pyelitis	Hemorr. into extensor muscle.
R17.2	1/25	2/7	1 hr.	##	0	Pyelitis Bilat.	Vaginitis.
R17.4	1/25	3/2	1 hr.	#	Absc.	No absc. in Section.	Pericarditis.
R17.8	2/8	2/13	1 hr.	###	0	0	
R17.12	2/8	3/24	1 hr.	##	0	?	
R 17	12/23	12/28	1 hr.	#	Absc.?	Medullary Absc.	Endometritis.
R 19	12/23	1/18	1 hr.	##	Absc.?	Medullary Absc.	

CONTROLS.

R17.3	0	0	1 hr.	0			Chilled.
R17.5	0	0	1 hr.	0			Chilled.
R17.9	2/8	3/2	0	#	0	Medullary Absc.	No Chilling.
R17.10	2/8	3/6	0	0	0	Medullary Absc.	No Chilling.

PROTOCOLLS OF EXPERIMENTS

R M 8.—Dec. 7. Injection into the ear vein of 1½ c.c. of a suspension in 5 c.c. NaCl of broth culture of B coli from S.

Wet thoroughly in cold water and held before the fan for 15 minutes.

Dec. 8. Urine examined: negative, temperature 40.6°. Diarrhea.

Dec. 9. Urine examined: negative. Diarrhea.

Dec. 11. Urine examined: negative.

Dec. 12. Many pus cells are seen in urine. Catheterized specimen contains many pus cells, groups of cells and epithelial cells. Temperature 39.6°. Urine cultured.

Dec. 13. Fewer pus cells in urine. Many epithelial cells. Temperature 39.5°.

Dec. 14. No pus cells in urine.

Dec. 18. No pus cells in urine.

Dec. 19. Urine negative.

Dec. 20. Urine negative.

Dec. 21. Many pus cells and groups of cells in urine. Temperature 39.4°.

Dec. 29. Urine shows 10-15 pus cells in low power field.

Dec. 29. P.M. Animal found dead and put on ice.

Dec. 30. *Autopsy*—The kidneys are apparently negative and there are no gross changes in any other organs.

Microscopic Examination—Left kidney. There is marked swelling of the convoluted tubules. No excess of cells is seen in the glomeruli. There are no areas of infiltration in the cortex or in the medulla. The pelvis is empty and the mucous membrane shows no infiltration with leukocytes. The right kidney resembles the left, except that there is more congestion of the cortex.

R. M. 9.—Dec. 13. Urine examined: Entirely negative. Injected intravenously with 1 c.c. heavy suspension of S. culture. Temperature before inoculation 38.4°. After inoculation the rabbit's fur was wet with cold water and animal then held before fan for 60 minutes. Temperature afterwards is 38.7°.

Dec. 14. Urine examined: negative, temperature 38.5°.

Dec. 15. Urine examined: negative.

Dec. 18. Urine examined: negative.

Dec. 19. Urine examined: Slight excess of pus cells; temperature 38.8°.

Dec. 20. Urine examined: About 6 pus cells to low power-field.

Dec. 21. Urine examined: An occasional pus cell. Expressed urine shows 5-10 pus cells and few casts.

Dec. 29. Urine examined: 3-4 pus cells.

Dec. 30. Urine examined: 5-6 pus cells.

Jan. 3, '17. Urine examined: negative.

Jan. 4. Urine examined: 4-5 pus cells.

Jan. 5. Urine examined: Negative.

Jan. 8. Urine examined: Slight excess of pus cells. An occasional hyalin cast.

Jan. 8. Sacrificed.

Autopsy—The kidneys are a brownish red color, perfectly smooth externally. On section of the kidney the relation of the cortex and medulla appears normal, but running down through the lower portion of the cortex and down into the medulla are numerous white opaque streaks, which in no part reach more than a third of the way through the medulla. The pelvis is normal. Culture of the bladder urine is negative.

Microscopic Examination—Kidney. The cortex shows numerous streaks rich in lymphocytes showing an excess amount of connective tissue, with destruction of the parenchyma. The process apparently is subacute or chronic. The cells are practically all of the lymphocytic type. The medulla shows marked congestion and a few dilated tubules and a few areas in which there is some increase in cells about the tubules. These are also the lymphocytic type. The pelvis is empty, and the wall shows no infiltration.

R M 10.—Dec. 12. Urine examined: Entirely negative. Injected intravenously with 1 c.c. heavy suspension of organism from broth culture of P.

Temperature before injection 38.5°. Wetted and held before fan for 10 minutes. Temperature after that is 38.9°.

Dec. 14. Urine examined: Negative. Temperature 38.9°.

Dec. 15. Urine examined: Negative.

Dec. 17. Dead.

Autopsy—Dec. 18. The bladder is distended with dark-colored urine. Examination of this shows epithelial cells, otherwise negative. The kidneys are negative. The stomach contains a

small ulcer. A purulent cholecystitis is present. The bladder is negative and also the culture.

Microscopic Examination—The kidney shows nothing abnormal in the cortex, medulla and pelvis.

R M 11.—Dec. 13, '16. Urine examined: Entirely negative. Injected intravenously with 1 c.c. heavy suspension of organism from broth culture of P. (B. coli.) Before injection the temperature 38.4°. Cold shower and then held before fan for 10 minutes. Temperature afterwards 38.8°.

Dec. 14. Urine examined: Negative. Temperature 38.4°.

Dec. 15. Urine examined: Negative.

Dec. 18. Urine examined: Negative.

Dec. 19. Urine examined: Negative.

Dec. 20. Urine examined: Negative.

Dec. 21. Urine examined: Negative.

Dec. 22. Urine examined: Negative, except for an occasional pus cell.

Dec. 28. Urine examined: 1-2 pus cells and several casts.

Dec. 29. Urine examined: 40-50 pus cells and several casts. Rabbit is sick.

Jan. 3, '17. Urine examined: Negative. Rabbit ill, died 30 minutes later. Sacrificed.

Autopsy—Some coccidiosis of the gall bladder. Otherwise entirely negative. Cultures of the gall bladder and bladder made.

Jan. 4. Culture of the bladder is negative. Culture of the gall bladder shows gram negative bacilli.

Microscopic Examination—In areas running through the lower portion of the cortex there is considerable increase in young connective tissue. The cortex is otherwise negative. The medulla and pelvis show nothing abnormal.

R M 12.—Dec. 22, '16. Urine normal. Injected intravenously with 2 c.c. of a suspension of B coli from urine of R M 8. Chilled for one hour after injection.

Dec. 23. Found dead in cage.

R M 13.—Dec. 22. Urine normal. Injected intravenously with 2 c.c. moderate suspension of B coli from urine of R M 8. Chilled for one hour after injection.

Dec. 29. 10-15 pus cells to low power field in urine.

Dec. 30. 1-2 pus cells in urine.

Jan. 3, '17. No pus cells in urine.

Jan. 4. An occasional pus cell in urine.

Jan. 5. An occasional pus cell in urine.

Jan. 8. Large excess of pus cells in urine—many groups. Temperature 39.5°. Sacrificed.

Autopsy—The kidneys are yellowish brown in color and both show scattered small hemorrhages over the surface. On section the cortex is of a grayish yellow color. In the medulla there are a number of opaque yellowish dots and streaks running toward the papilla. A portion of the medulla about the papilla is injected and shows numerous yellowish streaks running toward the point of the papilla. Culture of the bladder urine is negative. Autopsy is otherwise negative.

Microscopic Examination—Kidney. The cortex shows several areas of definite sclerotic changes with increase in connective tissue and infiltration with lymphocytes. The cortex is markedly congested and there is no increase in the number of cells in the glomeruli. Nowhere is there any acute infiltration in the cortex. In the medulla, on the other hand, in several streaks there is an acute infiltration with leukocytes and a breaking down of tissue. The pelvis is empty and the mucosa shows no infiltration with cells.

R M 14.—Dec. 22. Urine examined: Negative. Injected intravenously with culture from urine of R M 8 and chilled for one hour.

Jan. 3, '17. Urine examination: negative. Animal is very thin and looks ill.

Jan. 4. Urine contains 1-10 pus cells in low power field. Temperature 39.5°.

Jan. 5. No specimen of urine obtained. Temperature 38.6°. Left hind leg is tender. Sacrificed.

Autopsy—The findings are entirely negative except for fracture of the femur. Bladder urine contains epithelial cells and cellular debris. Culture of the urine is negative.

Microscopic Examination—No changes in the pelvis of the kidney or in the kidney substance were found.

R M 15.—Dec. 22. Urine examined: Negative. Injected intravenously with culture from urine of R M 8. Later chilled for one hour. Urine examined daily: negative entirely.

Dec. 29. Died.

Autopsy—Rabbit is very emaciated. On opening the peritoneum, the stomach and edge of the liver are adherent to the gall bladder by a thick yellowish-white exudate. The colon at the ileocecal valve is swollen and edematous and hemorrhagic for an extent of about 2 inches. The small intestine is filled with fluid content and considerable amount of air. The bladder is distended with clear urine. Kidneys are a brownish yellow color and smooth. On section the markings are normal. There is no evidence of a pathologic condition in the cortex, medulla or pelvis.

Microscopic Examination—The cortex shows a slight excess of eosinophiles in the glomeruli. There are no areas of infiltration in either the cortex or medulla. The pelvis is empty and the lining mucous membrane shows no infiltration.

R 8.—Dec. 23, '16. Urine normal. Intravenous injection of 5 c.c. of suspension of 2 tubes of 18-hour broth growth from F. Chilled 3 hours later for 1 hour.

Dec. 26. Animal is much emaciated and very weak. No urine obtained.

Dec. 27. Animal is weak. Expressed urine shows about 200 pus cells to low power field.

Dec. 28. Animal is stronger than on Dec. 27. No urine obtained. Sacrificed.

Autopsy—Animal is markedly emaciated. Temperature 37°. On opening the peritoneum the left kidney is seen to lie in groove of the spine. The bladder contains a small amount of urine. The stomach and colon are adherent to the gall bladder, which is covered with a dense whitish exudate. The heart and lungs are negative. The stomach and intestinal canal are also negative. The gall bladder is markedly thickened and the wall shows signs of coccidiosis. *Right kidney* is of a reddish yellow color and the surface is studded over with small yellow opaque areas, single and in large groups. The capsule strips readily. On section these opaque yellow areas extend down to the papilla, as opaque yellowish lines, single and in groups. The pelvis contains a thin purulent exudate but no macroscopic change. The ureter on the right side is slightly distended and a purulent material can be expressed that contains numerous cells and innumerable bacilli. The left kidney resembles in every way the right. The bladder

shows no hyperemia or other change. Cultures from both kidneys and bladder show gram negative bacilli.

Microscopic Examination—Kidney. The cortex is swollen and the convuluted tubules are markedly degenerated. There is a purulent exudate in the glomeruli and in the tubules. The tubules are filled with pus extending down into the medulla and in 3 or 4 areas are filled with pus cells, detritus and bacilli. At no place has a larger abscess been formed. Of especial interest is the purulent infiltration that the outer area of the pelvis in this and another section of the kidney show. The mucosa appears everywhere intact but below this layer there is a dense zone of pus cells varying somewhat in width and showing considerable nuclear fragmentation. In the mucosa a number of pus cells, apparently passing through, can be seen. The bladder is negative.

Ureter. The ureteral wall shows no infiltration. The lumen is filled with pus cells and many bacteria.

R 9.—Dec. 23, '16. Intravenous injection of 2 c.c. of 5 c.c. suspension of 2 tubes broth 18-hour growth from F. Chilled.

Dec. 24. Found dead.

R 10.—Dec. 23, '16. Urine normal. Intravenous injection of 2 c.c. of 5 c.c. suspension of 3 tubes 18-hour growth from S. Chilled.

Dec. 26. Animal is very weak—dying. Sacrificed.

Autopsy—Rabbit is considerably emaciated. On opening the peritoneum an enlarged gall bladder is seen glued down to the stomach surrounding the liver by a brownish opaque fibrinous exudate. The colon near the ileo-cecal valve is infiltrated and hemorrhagic. The kidneys are large, of a brownish-yellow color, and on section are very moist. The pelvis contains no excess of fluid and no pus. On tips of the papillae a few opaque yellowish streaks and dots are seen. The bladder is negative. The urine shows no pus cells. Blood culture is negative. Urine culture is also negative.

Microscopic Examination—Kidney. The cortex shows nothing abnormal. The medulla shows several areas in which the tubules are markedly distended and filled with granular material. The pelvis is empty and the lining shows no infiltration.

R 18.—Dec. 23, '16. Intravenous injection of 5 c.c. suspension of 3 tubes of 18-hour growth from S. (B coli). Chilled. Died a few minutes after chilling.

R 20.—Dec. 29. Intravenous injection of 24-hour broth growth from right kidney of rabbit 8. Found dead 4½ hours after injection.

R 21.—Dec. 29. Urine normal. Intravenous injection of 24-hour broth growth in 2 c.c. salt solution suspension from right kidney of rabbit 1. Four and one-half hours after injection thoroughly wetted and fanned for ½ hour in a cold room.

Dec. 30. Apparently dying. Died 26 hours after injection.

Autopsy—On opening the peritoneal cavity the bladder is enormously distended, injected and with small hemorrhages on surface. Also there is considerable edema, especially at the base of the appendix. Along the entire intestinal canal numerous small hemorrhages are seen subperitoneally. There is a large gland at the base of the appendix, enlarged and hemorrhagic. The appendix, when cut open, has a thickened yellow opaque wall with hemorrhages and a muco-hemorrhagic exudate in the interior. Small hemorrhages are seen on the surface of both kidneys which are greatly enlarged and soft. On section the cortex is thickened, yellow and small hemorrhagic areas are seen. The medulla near the cortex is a deep burgundy red color, apparently hemorrhagic, and this area runs down in a small streak to the papilla. The medulla below this streak is hyperemic, shows no yellow lines and does not appear otherwise abnormal. The pelvis shows nothing abnormal. The bladder appears normal.

The stomach has a small area near the pylorus which looks like superficial erosion, but is otherwise negative.

Culture of the left kidney shows pure culture of B. coli.

Microscopic Examination—Kidney. The cortex shows marked degeneration in the convoluted tubules and a slight excess of cells in the glomeruli. There is marked hyperemia of the cortex as well as of the medulla. In the medulla there are numerous areas in which there are hemorrhages. The pelvis of the kidney is empty. The mucous membrane shows no infiltration.

Cecum. Extensive hemorrhages through the entire musculature and to less extent into the mucosa are to be seen.

The bladder is normal.

Appendix. There are submucous hemorrhages. Marked inflammatory reaction and subserous masses of bacteria are to be seen, as well as blood pigmentation in the lymph centers.

R 22.—Dec. 29. Urine normal. Intravenous injection of 24-hour broth growth in 2 c.c. salt solution suspension from left kidney of rabbit 8.

One and one-half hours after injection thoroughly wet and fanned for ½ hour in a cold room.

Dec. 30. 40-50 pus cells in urine.

Dec. 31. 10 pus cells and 100 epithelial cells in urine.

Jan. 3, '17. Sacrificed.

Autopsy—The peritoneal cavity is clear and the appendix normal. There are a few small hemorrhages subserous in the cecum.

The gall bladder is normal. The stomach is negative and also the heart and lungs.

The kidneys are a yellowish brown color and uniform. The cortex is translucent and normal. The medulla is also normal. The pelvis is negative. The right ureter about 2 inches below the pelvis is definitely injected for about ½ inch. The bladder appears normal. Culture is negative.

Microscopic Examination—The kidney shows marked degeneration of the epithelium and in some areas just a narrow edge of epithelium remains.

The pelvis and the medulla are negative. The uterus shows acute inflammation of the mucous membrane and a leukocytic exudate in the crypts.

R 23.—Dec. 29. Urine normal. Intravenous injection of 24-hour growth broth in 2 c.c. salt solution suspension from left kidney of rabbit 8.

Four and one-half hours after injection, animal is thoroughly wet and fanned for ½ hour.

Dec. 30. 25-30 pus cells in urine.

Died about 4 P.M.

Autopsy—About 12 inches of jejunum is acutely inflamed. The bladder, appendix and stomach are negative. No culture taken.

Kidney is enlarged, the cortex swollen, yellow, in areas somewhat more opaque. The medulla is hemorrhagic in several areas, extending from the cortex to about half way to the papilla. Several such areas in the right kidney and one small one in the left.

Microscopic Examination—The kidney cortex shows marked

vacuolar degeneration of the epithelium, in some areas only a necrotic mass remaining in the center of the tubules.

The glomeruli are very hyperemic but show no excess of pus cells.

In a zone in the medulla there is very marked hyperemia and some extravasation of the blood into the tissues. In several tubules there is necrosis of the epithelium, with leukocytic infiltration and large masses of bacteria. This leukocytic infiltration extends for a considerable distance beyond the areas in which the bacteria are found.

The bladder shows nothing abnormal.

R 24—Dec. 31, '16. Expressed urine shows no pus cells.

2 P.M. Intravenous injection of 2 c.c. suspension of 24-hour plain broth growth from left kidney of R 21.

Thoroughly wet and fanned from 6 to 7 P.M.

Jan. 4, '17. No pus cells in urine.

Jan. 6. No pus cells in urine.

Jan. 12. No pus cells in urine.

Jan. 17. No pus cells in urine.

Jan. 18. Sacrificed.

Autopsy—In the cardiac end of the stomach there is a small depression measuring 2 or 3 mm. across, surrounded by a zone considerably thickened and raised above the general surface. The autopsy is otherwise negative. Culture of the bladder urine is negative.

Microscopic Examination—The kidney cortex is somewhat hyperemic but otherwise normal. The medulla is normal and the pelvis is empty. The epithelial lining shows no infiltration.

R 25—Dec. 31, '16. Expressed urine: 1st few gtts. 40-50 pus cells and several clusters. Next specimen expressed shows only 6-7 pus cells.

2 P.M. Intravenous injection of 2 c.c. suspension of 24-hour plain broth growth from left kidney of R 21.

Thoroughly wet and fanned from 6 to 7 P.M.

Jan. 1, '17. Expressed specimen contains 15 pus cells and many casts.

Jan. 4. Urine shows an occasional pus cell and much debris.

Jan. 6. Urine shows an occasional pus cell.

Jan. 12. No pus cells.

Jan. 17. No pus cells.

Jan. 18. Sacrificed.

Autopsy—On the posterior surface of the right kidney there is a marked depression densely adherent to the capsule. On section the cortex at this point is almost completely gone and is made up of a dense connective tissue. The autopsy is otherwise negative. Culture of the urine is negative.

Microscopic Examination—The kidney shows marked degeneration of the epithelia and occasional areas of necrosis. The glomeruli show nothing abnormal. There are no areas of infiltration anywhere.

R 26—Dec. 31, '16. Expressed specimen of urine shows no pus cells.

2 P.M. Intravenous injection of 2 c.c. suspension of 24-hour plain broth growth from right kidney R 8.

Thoroughly wet and fanned from 7 to 8 P.M.

Jan. 1, '17. Expressed specimen of urine shows 25-30 pus cells and numerous large gram casts.

Jan. 2. Expressed specimen of urine shows 2-3 pus cells.

Jan. 4. An occasional pus cell in urine.

Jan. 6. Large excess of pus cells in urine.

Jan. 8. No excess of pus cells in urine but an occasional cast.

Jan. 12. No excess of pus cells in urine.

Jan. 18. No pus cells in urine. Sacrificed.

Autopsy—The right lower lobe of the lung is completely consolidated and covered with a purulent exudate. On section this is made up of a number of thickened abscess cavities. The lung just above is a yellowish gray color and consolidated. The kidney shows nothing abnormal externally or on section. Otherwise the autopsy is entirely negative. The culture of the bladder urine is negative.

Microscopic Examination—Kidney. The cortex is practically normal. The medulla shows numerous areas of infiltration with lymphocytes involving in some areas 2 or 3 and in others as many as 6 or 8 tubules. In the center of these areas there seems to be a slight proliferation of the epitheliod cells. The pelvis shows nothing abnormal, except where these areas come in contact with the surface, where there is a lymphocytic infiltration of the

mucous membrane. The lung shows extensive cavities. Search for tuberculosis bacilli in the abscess wall is negative.

R 27—Dec. 31. Expressed specimen of urine shows no pus cells.

2 P.M. Intravenous injection of 4 c.c. suspension of 24-hour plain broth growth from right kidney R 8.

Thoroughly wet and fanned from 7 to 8 P.M.

Jan. 1, '17. Found dead.

Autopsy—Both kidneys showed hemorrhages in the medulla. The bladder is distended with urine but otherwise negative. The cortex is markedly thickened, yellow and opaque. The medulla is markedly injected. The pelvis shows nothing abnormal.

Microscopic Examination—The kidney cortex, medulla and pelvis show nothing abnormal.

R 28.—Dec. 31. Expressed specimen shows no pus cells.

2 P.M. Intravenous injection of 6 c.c. suspension of 24-hour growth plain broth from kidney R 8.

Thoroughly wet and fanned from 7 to 8 P.M.

Jan. 1, '17. Animal is very sick—diarrhea. Much pus in stool. In expressed urine there are 5 pus cells.

Jan. 5. Animal died.

Autopsy—Jan. 5. Rabbit in rigor mortis. Body is not yet entirely cold. On opening the peritoneum the surface is everywhere smooth. The appendix, cecum, gall bladder, bladder are all normal. The kidneys are a brownish red color but show nothing unusual externally. On section the cortex is somewhat thickened. The medulla and cortex are otherwise negative. The pelvis is somewhat more opaque than usual but shows nothing abnormal. The ureters are negative. The right lung is covered with a fibro-purulent exudate. The middle lobe is consolidated, its edge of a red color. On section the red, wedge-shaped area is entirely solid and of a dark red color. Remainder of the lobe is air containing except for a few hemorrhagic spots.

The pericardium also contains a small amount of fibro-purulent exudate.

The heart is negative on opening. The left lung is normal.

The urine from the bladder contains many hyalin casts but no pus cells.

Microscopic Examination—The cortex shows marked degeneration of the epithelium and in some areas complete necrosis. There is no increase of cells in the glomeruli. Along the collecting tubules, extending down into the medulla, there is diffuse infiltration with polymorphonuclear cells, which at no place has led to abscess formation, but in some places to small collections of polymorphonuclear leukocytes. The pelvis shows nothing abnormal. The lung shows a hemorrhagic infarct with extensive bronchitis.

R 17.1.—Jan. 25. Expressed specimen of urine is negative.

2:30 P.M. Intravenous injection of 20-hour plain broth culture of streptococcus from L.

6:30 P.M. Thoroughly wetted and fanned until 7:30 P.M.

Jan. 26. Urine examed: Negative.

Jan. 27. Urine examined: 1 or 2 groups of cells and an occasional cast.

Jan. 29. Urine examined: Negative.

Jan. 30. Urine examined: Large excess of pus cells. Temperature 40.15°.

Smear of urine: A few gram positive diplococci.

Jan. 31. Urine examined: Excess of pus cells, largely in groups. Temperature 39.6°.

Feb. 1. Urine examined: Excess of pus cells. Smear shows a large number of gram positive diplococci. Temperature 39.5°. No albumin.

Feb. 2. Urine examined: Large excess of pus cells and groups of cells. Urine cloudy acid.

Feb. 5. Innumerable pus cells in low power field. Stained smear shows countless gram positive cocci in pairs and chains. Albumin —, acid.

Feb. 6. Urine examined: Countless pus cells, flakes of pus. Sacrificed.

Autopsy—Animal is in good physical condition. On opening the peritoneal cavity there is no evidence of coccidiosis. The peritoneum is everywhere smooth. The bladder is slightly distended. The ureters and kidney appear normal externally. There are hemorrhages into the extensor muscle on both thighs. The heart shows nothing unusual. The lungs are negative. The gall bladder, stomach and intestinal canal show nothing abnormal. The *left kidney* is of a reddish brown color, smooth and no

hemorrhages are to be seen externally. The ureter appears practically normal. On pressure pus can be expressed from the cut end. On opening the kidney, a thin liquid pus exudes from the cut section. On section the kidney epithelium seems rather yellowish and opaque and the medulla is light pink in color. The pelvis is distended with thin pus. There is no evidence anywhere of abscess in the kidney substance. The pelvis is not injected.

Right Kidney—The ureter is normal and no pus can be expressed, only epithelial debris. The kidney surface is everywhere smooth. On section the cortex is a grayish, somewhat opaque color. The pelvis contains no pus. The kidney appears normal otherwise. Bladder shows nothing abnormal.

Cultures from the bladder urine give a streptococcus. Heart blood culture is negative.

Microscopic Examination—Left kidney. There is considerable cloudy swelling of the convoluted tubules and an occasional space is filled with serum. The glomeruli contain no excess of cells. The medulla shows nothing abnormal. The pelvis of the kidney shows only a very small amount of free exudate in the lumen but very marked infiltration with polymorphonuclear leukocytes in the lining membrane. In some portions the eosinophile leukocytes are crowded together in dense masses, frequently lying as a solid layer, directly below the mucosa. The right kidney shows parenchymatous degeneration of the convoluted tubules but is otherwise negative. The left ureter shows nothing abnormal. The bladder is negative.

R 17.2.—Jan. 25. Expressed specimen of urine is negative.

2:20 P.M. Intravenous injection of 20-hour plain broth culture of streptococcus from L.

6:30 P.M. Thoroughly wetted and fanned until 7:30 P.M.

Jan. 26. Urine examined: No pus cells but 10-12 granular casts in low power field.

Jan. 27. Urine examined: 8-10 pus cells per low power field; no casts.

Jan. 29. Urine examined: 20-25 pus cells per low power field; an occasional cast.

Jan. 30. Urine examined: Large excess of pus cells. Temperature 39.6°.

Smear of urine shows no bacteria.

Jan. 31. Urine examined: Large excess of pus cells. Temperature 39.5°.

Feb. 1. Urine examined: Excess of pus cells. A few flakes of pus. Smear shows no bacteria. Temperature 39.0°; no albumin.

Feb. 2. Urine is very clear. One or two pus cells seen in low power field; acid.

Feb. 5. Urine is cloudy and shows an excess of pus cells: one large group and relatively few single cells. Stained smear shows 5-6 gram positive diplococci—only bacteria present.

Feb. 6. Urine shows a large excess of pus cells and many streptococci.

Feb. 7. Urine loaded with pus cells and streptococci. (Expressed specimen.) Sacrificed.

Autopsy—On opening the peritoneal cavity the abdominal organs appear entirely negative. The bladder is slightly distended with urine which contains few pus cells and a large number of casts. There is a large amount of pus in the vagina. Smears from the bladder contain many streptococci. On section of the ureter pus can be expressed. There is no pus in the pelvis of the kidneys. Culture of the heart's blood and bladder both show gram positive streptococci.

Microscopic Examination—Kidney. The cortex and the medulla show nothing unusual. The pelvis is free from exudate except an area where there are masses of pus cells, detritus and blood corpuscles. The mucous membrane lining the wall is infiltrated with polymorphonuclear leukocytes—in some areas very dense and in others only an occasional leukocyte can be seen lying between the epithelial cells. The ureter shows nothing. The bladder shows a normal intact mucous membrane.

R 17.4.—Jan. 25. Expressed specimen of urine is negative.

2:30 P.M. Intravenous injection of 20-hour plain broth culture of streptococcus from L.

7:30 P.M. Thoroughly wetted and fanned until 8:30 P.M.

Jan. 26. Expressed specimen of urine: In low power field shows 5-6 pus cells and 1-3 granular casts.

Jan. 27. Urine in low power field shows 5-6 pus cells and 1-3 granular casts.

Jan. 29. Urine examination: Negative.

Jan. 30. Urine examination: Temperature 39.7°.

Jan. 31. Urine examination: Negative. Temperature 39.5°.

Feb. 1. Urine examination: Negative. Temperature 39.9°. No albumin.

Feb. 2. Urine examination: Negative. Urine cloudy but debris clears up with dilute acetic acid. Urine is faintly acid.

Feb. 5. Urine shows a slight excess of pus cells. Much debris is in expressed urine, brown, probably fecal. Stained smear shows no bacteria. Acid.

Feb. 6. Urine shows a few pus cells but is otherwise negative and there are no bacteria.

Feb. 7. 4-5 pus cells are seen in low power field.

Feb. 8. Urine is negative.

Feb. 9. 4-5 pus cells are seen in low power field; otherwise negative.

Feb. 10. Urine is negative, except for large masses of amorphous precipitate. Acid clears with acetic.

Feb. 12. Urine shows about 30 pus cells in low power field. No bacteria in stained smear. A few casts.

Feb. 13. No excess of pus cells in urine.

Feb. 14. No pus cells in urine.

Feb. 15. About 30 pus cells in low power field. No casts.

Feb. 24. No pus cells or casts in urine.

Feb. 27. Urine negative.

April 2. Died.

Autopsy—The bladder is greatly distended with urine which contains 10-12 casts, albumin but otherwise negative. The kidneys are soft and red. The right kidney is negative and the pelvis is smooth. The left kidney has one small abscess extending from the cortex almost to the medulla—about .5 mm. in diameter. The pelvis is everywhere smooth. The stomach, appendix and intestines are negative. Heart shows a pericarditis with fibrinous exudate but is otherwise negative. The lungs appear hemorrhagic in areas and consolidated in parts. The liver shows slight coccidiosis but the gall bladder appears normal. Cultures from the bladder, heart blood and abscess of the left kidney are all sterile.

Microscopic Examination—The kidney shows marked degeneration of the convoluted tubules. The glomeruli show an excess of eosinophile cells. In some areas there are small collections

of eosinophile cells outside of the glomeruli. The cortex is otherwise normal. The medulla is rather hyperemic, especially at the tip of the papillae and shows no areas of infiltration. The pelvis is empty. The epithelium shows no signs of infiltration.

R 17.8.

Feb. 8. Urine examined: Negative, acid, albumin —.

4:30 P.M. Injected same as R 17.10, streptococci from L. Wetted and chilled for 1 hour before fan 5½ hours after injection. Animal ill after the chill.

Feb. 9 A.M. Animal is better. Urine examined: Large number of epithelial cells and some epithelial casts. No pus cells.

Feb. 10. Urine shows a large excess of pus cells—about 50-60 to low power field. Also an excess of casts.

Feb. 12. Urine contains shreds of pus and large excess of single cells and many casts. Smear shows gram positive streptococci. Animal is weak on hind legs.

Feb. 13. Large number of pus cells in urine. Casts+. Sacrificed.

Autopsy—Localized peritonitis around the gall bladder involving under surface of the liver and stomach is seen. Submucosal hemorrhages are scattered over the stomach. The appendix is full of fecal material but is otherwise negative. The bladder shows several hemorrhages. The vagina and urethra are negative. Material expressed from the ureters contains casts and epithelial cells but few pus cells. The kidneys are entirely negative.

The bladder urine contains casts and some pus cells but not a large number. Culture of bladder urine in dextrose broth shows mixed streptococcus and gram negative bacilli.

Microscopic Examination—The bladder mucous membrane is intact and there is no infiltration. The appendix shows some necrosis of the lymph follicle. The gall bladder shows some abscesses in the wall.

The kidney, except for slight parenchymatous degeneration of the cortex, shows nothing abnormal.

R 17.12.

Feb. 8. Urine examined: Negative, no albumin, acid.

Feb. 8. Injected same as R 17.10 with streptococcus R 17.1 from L.

Wetted and chilled for 1 hour before fan 5½ hours after injection.

Feb. 9. Urine examined: Negative, except for much amorphous debris.

Feb. 10. Urine examined: Negative.

Feb. 12. Urine loaded with pus cells, single and in groups. Smear shows a number of streptococci.

Feb. 13. Urine shows no pus cells.

Feb. 14. Urine shows no pus cells.

Feb. 15. Urine shows no pus cells or casts.

Feb. 16. Urine shows about 30-40 pus cells in low power field. One large group of cells. Smear shows no bacteria.

Feb. 17. 10-12 pus cells in urine in low power field.

Feb. 19. 50-60 pus cells in urine in low power field. Much amorphous debris.

Feb. 20. Large excess of pus cells and groups of cells in urine. Stained smear shows a few gram positive diplococci but no other organism.

Feb. 21. About 50 pus cells in low power field. A few in groups. Smear shows no bacteria.

Feb. 24. Urine loaded with pus cells and one or two casts.

Feb. 26. Urine loaded with pus cells and 20 casts in low power field. Albumin absent. Smear shows no bacteria. Temperature 39.00°.

Feb. 27. Urine shows no pus cells or casts.

April 2. Slight excess of pus cells. Albumin present, acid.

April 7. Large excess of pus cells and groups of cells in urine. Smear shows many streptococci in chains but no other organisms.

April 8. Urine is loaded with pus cells.

April 9. Urine loaded with pus cells, and excess of casts. Smear shows many streptococci but no other organisms.

April 12. Urine loaded with pus cells and several casts. Smear shows many streptococci but no other organisms.

April 14. Loaded with pus cells and casts. Smear shows just streptococci.

April 20. 50-60 pus cells and 10-12 casts.

April 24. Died.

Autopsy—The bladder is half full of urine, containing 4-5 pus cells and much debris. The gastro-intestinal tract is negative

except for some white spots on the appendix. *Kidneys.* The pelvis contains no pus. Some dark spots are seen on the kidneys, probably not acute. Otherwise the autopsy is entirely negative.

No cultures made.

Microscopic Examination—The kidney cortex is slightly congested but otherwise normal. The medulla is also congested but shows no areas of infiltration. The pelvis is empty. There is no infiltration of the mucous membrane. The ureter is empty and shows no infiltration of the wall. The appendix shows massive necrosis in the central portion of the lymphoid follicles. A large number of bacilli can be seen lying in the lymphoid tissue.

R 17.—Dec. 23. Urine normal. Intravenous injection of 2 c.c. of 5 c.c. suspension of 3 tubes of 18-hour growth from L. Chilled.

Dec. 27. Expressed urine shows 30-40 pus cells to low power field. Aborted.

Dec. 28. Expressed urine apparently is pure pus. Temperature 40°.

Animal sacrificed.

Autopsy—Well developed female rabbit. On opening the peritoneal cavity, the uterus is enlarged and injected. The bladder is full of urine which contains no pus. The heart, lungs, liver, stomach and appendix are negative. The kidney has small reddish depressions on the surface. On section there is a small red streak in the medulla. The pelvis is negative. The uterus is ulcerated in areas. The vagina is filled with a thick, yellow-green pus. The pus shows no bacteria.

Microscopic Examination—Right Kidney. The convoluted tubules show considerable swelling and evacular degeneration. The glomeruli are normal. The medulla shows no infiltration or hyperemia. *Left Kidney.* In the cortex the swelling of the convoluted tubules is very marked. In the upper portion of the medulla there are several areas which are densely infiltrated. The outlines of the tubules are obliterated. There are some polymorphonuclear leukocytes but principally cells of the lymphocytic type. Further down in line with these areas there are other zones of infiltration. The pelvis contains an hemorrhagic exudate in the lumen. The mucous membrane shows slight infiltration with lymphocytes.

R 19.—Dec. 23. 2 c.c. intravenous injection of 5 c.c. of a suspension culture of 3 tubes of 18-hour growth from L. (Streptococci.)

Chilled.

Dec. 27. No urine obtained.

Dec. 28. 3-8 pus cells obtained.

Dec. 29. 3-4 pus cells in urine.

Dec. 30. 40-50 pus cells in urine.

Jan. 4, '17. Large excess of pus cells and some groups of cells.

Jan. 5. Slight excess of pus cells.

Jan. 6. About 15-20 pus cells to low power field.

Jan. 8. About 30 pus cells to low power field.

Jan. 17. About 60 pus cells to low power field.

Jan. 18. Sacrificed.

Autopsy—The organs are all negative except the stomach just at the pylorus, an area in which there are 3 or 4 small hemorrhages—one about 2 mm. in diameter. The kidneys are smooth on surface, brownish red color. On opening the right kidney, it appears normal. The left kidney is opened longitudinally. There are seen a number of streaks running from the cortex through the medulla about to the tips of the papillae. The autopsy is otherwise negative. Examination of the bladder urine shows about 60 positive pus cells per low power field, single and in groups. Kidney culture of the bladder urine shows gram positive diplococci and short chains.

Miscroscopic Examination—In the cortex and extending down into the medulla there are some areas of infiltration in which the kidney structure has been destroyed; 5 or 6 such areas can be seen in a single section. The pelvis is empty. The mucous membrane shows no infiltration, except where the infiltration of the medulla comes in contact with the surface.

R 17.3.—Control. Jan. 25. Expressed specimen of urine is negative. No injection made.

6:30 P.M. Thoroughly wetted and fanned until 7:30 P.M.

Jan. 26. Urine examined: Negative.

Jan. 27. Urine examined. Negative.

Jan. 29. Urine examined: Negative.

Jan. 30. Urine examined: Negative. Temperature 39.5°.

Jan. 31. Urine examined: Negative. Temperature 39.7°.

Feb. 1. Urine examined: Negative. Temperature 38.8°. No albumin.

Feb. 2. Urine examined: Negative, crystal clear, acid.

Feb. 7. Urine examined: No cells.

Negative entirely.

R 17.5.—Control. Jan. 25. Expressed specimen of urine is negative. No injection made.

7:30 P.M. Thoroughly wetted and fanned until 8:30 P.M.

Jan. 26. Expressed specimen of urine: No pus cells.

Jan. 27. Expressed specimen of urine: No pus cells.

Jan. 29. Urine examination: Negative.

Jan. 30. Urine examination: Negative. Temperature 39.4°.

Jan. 31. Urine examination: Negative. Temperature 39.5°.

Feb. 1. Urine examination: Negative. Temperature 39.2°; no albumin.

Feb. 2. Urine examination: Negative. Acid cloudy with debris. Clears up with dilute acetic acid.

Feb. 7. Urine examination: Negative; trace of albumin.

R 17.9.—Control. Feb. 8. Urine examined: Negative.

Injected same as R 17.10—streptococci from L.

Feb. 9. Urine examined: About 12 granular casts in low power field and 5-6 pus cells.

Feb. 10. Diarrhea. No specimen obtained.

Feb. 12. Urine negative.

Feb. 14. Urine shows about 20 granular casts in low power field and 5-6 pus cells.

Feb. 15. One or two granular casts.

Feb. 16. Urine examination: Microscopic negative. Smear shows no bacteria.

Feb. 20. Urine examination: About 10 casts, granular and hyalin to low power field. No pus cells.

Feb. 24. Urine shows about 5-10 pus cells, no casts to low power field, acid.

April 2. No urine obtained. (Pus exuding from the vagina.)

April 6. Dying. Sacrificed.

Autopsy—Made immediately. Female rabbit. The bladder urine contains many pus cells, single and in groups, and many casts. The kidneys are negative and also the stomach, appendix,

lungs, heart, etc. Marked coccidiosis of the gall bladder and liver is seen. The bladder is edematous, congested, and shows a slight exudate. Culture of bladder urine gives mixed growth of gram positive diplococci and gram negative bacilli.

Microscopic Examination—The bladder shows nothing abnormal. The cortex of the kidney shows considerable dilatation of the collecting tubules with atrophy of the walls. The glomeruli are crowded full of eosinophile cells and there is also an excess of eosinophile cells in the other portions of the cortex.

The medulla is hyperemic and shows numerous areas that are densely infiltrated with polymorphonuclear leukocytes, principally of the eosinophile type. The pelvis is empty and the mucous membrane shows no infiltration.

R 17.10.—Control. Feb. 8. Urine examined: negative, except a trace of albumin, acid. Injected intravenously with 1 c.c. of a suspension of 48-hour culture from bladder of R 17.1. (Streptococcus from L.)

Feb. 9. Urine examined: About 10 casts to low power field, but otherwise negative.

Feb. 10. Urine examined: Negative, no casts.

Feb. 12. Urine examined: Negative, no casts.

Feb. 14. Urine examined: One or two casts, but otherwise negative.

Feb. 15. Urine examined: One or two casts.

Feb. 16. Urine examined: Microscopic negative. Smear shows no bacteria.

Feb. 20. Urine examined: About 10-12 pus cells to low power field and 2-3 casts.

Feb. 24. Urine examined: About 10 casts to low power field and 2 or 3 pus cells, acid.

April 2. Large number of granular and hyalin casts, acid, albumin —.

April 6. Died.

Autopsy—Male rabbit. The bladder urine contains a large amount of cellular debris, epithelial cells, and casts. The kidney pelves are smooth and nothing abnormal is seen. There is a hemorrhage on a portion of the small intestines. The stomach and duodenum are negative. There is some coccidiosis of the gall bladder and liver. The heart and lungs appear normal and also the appendix. The bladder is negative and the urethra is

congested and hemorrhagic. Culture of the bladder urine is negative.

Miscroscopic Examination—Kidney. There are numerous small longitudinal abscesses in the medulla and an excess of eosinophiles in the glomeruli.

The mucosa of the pelvis shows some areas slightly infiltrated with cells. The lumen is empty. In the ileum there are several large hemorrhages into the submucosa.

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THE BACTERIOLOGY OF THE URINE IN CHILDREN WITH VULVOVAGINITIS

WITH A REPORT OF TWO CASES OF GONOCOCCUS CYSTITIS *

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The conception of an ascending route of infection in cystitis of infancy has heretofore largely rested on the unproved assumption of a constantly contaminated urethral tract. Such a hypothesis served to explain the predominance of cystitis in female infants. Helmholz and Beeler[1] in a recent study of the bacteriology of the urine in normal children, and of the urine from children with extra-urinary infections, established conclusively that the organisms of the colon group are not normal inhabitants of the female urethra. They further demonstrated that in those instances of extra-urinary infection in which organisms were found in the urine, these were present in such small numbers that they could not be considered other than accidental contaminations from the urethra.

If the incidence of cystitis were subject to local factors, then any condition which increased such local factors should militate against a sterile urinary tract. It therefore seemed important to study the bacteriology of the urine in children with vulvovaginitis. For we have in this disease inflammatory changes the contiguity of which to the urethra might conceivably produce a *locus minoris resistentiae*, which would favor contamination of the urinary tract with resulting cystitis. The bacteriology of the urine from eighteen unselected patients with chronic gonococcus vulvovaginitis was studied. These patients were all over 2 years of age. Catheterized specimens were obtained and cultures made, using the technic and method described by Helmholz and Beeler.

Two specimens were obtained from each patient, the first representing approximately the flora of the urethra, the second that of the bladder. A portion of the first specimen was examined for albumin, another part centrifuged and examined in stained smears for bacteria and unstained for cellular elements.

At the time of catheterization, thirteen of these eighteen patients presented more or less vaginal discharge, and all of the eighteen had leukocytes in their vaginal smears. Only five showed gonococci in the vaginal smears at the time of catheterization. None of these eighteen

* From the Otho S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital, Chicago.

1. Beeler, C., and Helmholz, H. F.: Bacteriology of the Urine in Healthy Children Suffering from Extra-Urinary Infections, Am. Jour. Dis. Child., 1916, **12**, 345.

TABLE I. BACTERIOLOGY OF THE URINE IN CASES OF VULVOVAGINITIS

Case No.	Vulvovaginal Findings: Discharge	Vulvovaginal Findings: Leukocytes	Vulvovaginal Findings: Gonococci	Urine Examination: Albumin	Urine Examination: Microscopic	Cultures of Specimen 1: Colonies per C.c.	Cultures of Specimen 1: Types of Organisms	Cultures of Specimen 2: Colonies per C.c.	Cultures of Specimen 2: Types of Organisms	Remarks
1	+	+	+	—	Negative	10	Staphylococcus albus; diphtheroid bacillus	Less than 1	Gram + coccus	Diphtheroid bacillus, which constituted majority of colonies, appeared only on a blood agar plate
2	+	+	—	—	Negative	0		0		
3	+	+	—	—	Stained sediment shows large gram + coccus	Innumerable	Staphylococcus albus	Innumerable	Staphylococcus albus	Identical findings on recatheterization 18 days later
4	+	+	+	—	Negative	0		Less than 1	Bacillus subtillis	Only 1 colony present, this not appearing until 6th day on blood agar plate
5	—	+	—	Faintest trace	Negative	Less than 1	Bacillus subtillis	0		Only 1 colony present, this on the blood agar plate
6	—	+	—	—	Negative	3	Staphylococcus albus	10	Staphylococcus albus	Specimen 2 inoculated in deep dextrose blood agar tube only
7	+	+	—	—	Negative	Less than 1	Diphtheroid bacillus; gram + coccus	Less than 1	Diphtheroid bacillus	
8	+	+	—	—	Negative	3	Staphylococcus albus; gram + diplococci	2	Gram + coccus; gram + diplobacillus	
9	+	+	+	—	Negative	Less than 1	Bacillus subtillis	1	Staphylococcus albus; gram + diplococcus	
10	+	+	—	—	Negative	0		0		
11	—	+	—	—	Negative	6	Gram + diplococcus	6	Gram + diplococcus	
12	—	+	—	—	Negative	1	Gram + diplobacillus; Staphylococcus aureus; diphtheroid bacillus	10	Gram + diplococcus	Only medium showing growth from Specimen 2 was deep tube of dextrose blood agar
13	+	+	—	—	Stained sediment shows gram — coccus. No cellular elements	0		0		
14	—	+	—	—	Negative	3	Diphtheroid bacillus; gram + diplobacillus	0		
15	+	+	—	—	Negative	2	Diphtheroid bacillus; gram + diplococcus	0		
16	+	+	—	—	Negative	0		0		Only Specimen 2 cultured
17	+	+	+	+	Large number of pus cells; stained sediment shows leukocytes with gram — diplococci	Less than 1	Staphylococcus albus	Less than 1	Gram + diplobacillus	Urine milky; contains flocculi; acid reaction
18	+	+	—	+	Large number of pus cells; stained sediment shows leukocytes with gram — diplococci	Less than 1	Gram — short diplobacillus; would not grow in subcultures	0		Urine milky; contains flocculi; acid reaction

patients, however, had had vaginal smears negative for gonococci for any three consecutive weeks (a requirement prior to discharge of such patients from this hospital).

The bacteriologic findings were as follows: (Table 1) Specimen 1 was sterile in five instances, in ten instances gave less than 3 colonies per cubic centimeter, in one instance 6 colonies per cubic centimeter, in one instance 10 colonies per cubic centimeter, and one showed innumerable colonies. Specimen 2 was sterile in eight instances, gave 1 or an average of less than 1 colony per cubic centimeter in five instances, 2 colonies per cubic centimeter in one instance, 6 colonies per cubic centimeter in one instance, 10 colonies per cubic centimeter in two instances, and one showed innumerable colonies. From the same patient whose urine gave a profuse growth of a *Staphylococcus albus* in both Specimens 1 and 2, subsequent catheterization and cultures brought identical findings. This urine showed no other abnormalities, nor did the particular patient exhibit any subjective evidence of urinary disturbance.

TABLE 2.—Bacteriology of the Urine After Recatheterization

Case No.	Vulvovaginal Findings			Urine Examinations		Cultures	
	Discharge	Leukocytes	Gonococci	Albumin	Microscopic	Colonies per C.c.	Types of Organisms
1	—	+	+	—	Negative.....	2	Diphtheroid bacillus
2	—	+	—	—	Negative.....	2	Staphylococcus albus
3	+	+	—	--	Negative for cellular elements	Innumerable	Staphylococcus albus
4	+	+	+	—	Negative.....	0	
5	—	—	—	—	Negative.....	Less than 1	Diphtheroid bacillus
6	—	+	+	—	Negative.....	0	
7	—	+	—	--	Negative.....	0	
8	—	+	—	—	Negative.....	1	Staphylococcus
9	—	+	—	—	Negative.....	Less than 1	Gram negative bacillus
10	+	+	—	—	Negative.....	Less than 1	Bacillus, gram ±

The character of the organisms obtained were very similar to those reported in the series of normals by Helmholz and Beeler, namely, gram-positive cocci and diphtheroid bacilli. In no instance was a gram-negative bacillus encountered.

In Cases 17 and 18, however, as shown in Table 1, the sediment, even uncentrifuged, showed masses of pus cells, with occasional leukocytes filled with gram-negative, biscuit-shaped diplococci. These two instances will be discussed at greater length in the latter part of this article.

After an interval of several weeks, ten of the patients, who were kept constantly under observation in the interval, were again catheterized, slightly varying the methods of study. In the series of recatheterizations (Table 2; the numbers correspond with case numbers in Table 1) Specimen 1 was used for centrifugation and microscopic study but was not cultured. Specimen 2 was planted on plates of lactose litmus agar and ascites dextrose agar, the former to make identification of any of the colon group easier, the latter for growth of the gonococcus. The total result, practically negative findings in the series of recatheterizations, is a circumstance of striking importance. It is noted that in not a single instance, as shown either by the clinical urinary analysis or by the bacteriologic findings, does the recatheterization reveal any urinary infection which might be conceived as following either the vulvovaginal infection or the first catheterization per se. In only one instance, the patient with staphylococcus bacteriuria mentioned previously, were the bacteria present in such numbers that they could not be considered as accidental contamination from the urethra. Of the ten specimens, three were sterile, all the others but one showing less than 2 colonies per cubic centimeter. The few organisms encountered were either staphylococci or diphtheroid bacilli, except in Case 9, in which the ascitic dextrose agar plate showed one colony, a gram-negative bacillus.

Comparing the results of the present series with those reported by Helmholz and Beeler in children over 2 years of age, a difference is only noted in regard to Specimen 1. Of their series, 83 per cent. showed 1 colony or less per cubic centimeter. In the present series this percentage is 58, giving a smaller number of practically sterile urines in those obtained from patients with vulvovaginitis. In the comparison of the results obtained from cultures of Specimen 2, this difference disappears. Of their series, 73 per cent. showed either a sterile urine or less than 1 colony per cubic centimeter. In the present series 72 per cent. showed the same findings. The negligible difference in results with Specimen 1, is easily explained, in that it is quite conceivable that during a vulvovaginitis, the distal end of the urethra may harbor more bacteria than normally, and Specimen 1 of these respective series represents this flora as well as that of the deep urethra. Specimen 2, however, representing the bladder flora, shows the same comparative absence of organisms in the urine of patients with vulvovaginitis as was demonstrated in that obtained from normal children.

GONOCOCCUS CYSTITIS

Notwithstanding the frequency of gonococcus vulvovaginitis in childhood, particularly as met with in hospital practice, and the numerous reports that have been published regarding this disease, one is

struck with the very meager data at hand regarding gonococcus cystitis in childhood. Statistical tables in the majority of investigations are conspicuously silent on this complication, many not mentioning it, others noting a rare case with few details in a large series of observations. Welt-Kakels[2] notes that gonococcus cystitis is but very rarely observed, having seen only one case in a series of 190 children with vulvovaginitis. Edith Spaulding,[3] reporting 5 cases of cystitis among 74 children with vulvovaginitis, says: "We believe cystitis is much more common than is realized, but as the urine is not examined in every case, it is often not discovered." Neither of these observers states how the diagnoses were reached in their cases. It should be remarked that even with urinalyses, cystitis may be overlooked, the cellular elements encountered being attributed to the vaginal discharge rather to coexistent cystitis. This is probably the most frequent source of error and may account for the infrequency of hospital diagnoses of this condition. In this connection it might also be stated that presence of leukocytes in a vaginal smear, sometimes wrongly interpreted as evidence of a latent vaginitis, may occasionally be due to an undiagnosed cystitis of *Bacillus coli* origin.

In the present series of vulvovaginitis cases two instances of gonococcus cystitis were encountered. It is extremely interesting that both of these patients had had previous infection of the urinary tract, one having entered the hospital with a colon cystitis, the other with a mild nephritis associated with a colon bacilluria, vulvovaginitis developing in both at a later time. Their persistent cystitis had not aroused any suspicion of the true infection until they were catheterized for the purpose of the previous study. Like many other patients with cystitis, there were no subjective complaints. On this catheterization and on later catheterization, the urine obtained in both instances was of a milky character and held suspended numerous fine flocculi. Uncentrifuged smears showed innumerable pus cells, many exhibiting the characteristic inclusion of gram-negative diplococci. It is of course impossible to state when the *Bacillus coli* had been displaced by the gonococcus as the infecting organism. One of these patients was cystoscoped by Dr. Herman L. Kretschmer. The bladder showed a cystitis at the trigonum, the usual pathologic picture of such infections. The ureteral orifices as seen with the plain cystoscope appeared normal.

These instances afford another possible explanation for the occasional protracted case of vulvovaginitis that is refractory to treatment. Such a bladder mucosa might act as a source of reinfection of the vaginal tract.

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2. Welt-Kakels: New York Med. Jour., 1904, **80,** 780.
3. Spaulding, E. R.: Vulvovaginitis in Children, Am. Jour. Dis. Child., 1913, **5,** 248.

The difficulty of cultivation of these organisms from the urine has been noted by Colombini[4] and others. Whether this same slow growth of the gonococcus accounts for the apparent rarity of this organism as a cause of cystitis in children, may only be conjectured. It has been shown by several investigators that the mere presence of the gonococcus in the bladder will not produce a cystitis. In these particular instances the previous infection by the *Bacillus coli* may have produced changes in the bladder mucosa favorable for subsequent infection by the gonococcus.

SUMMARY

In eighteen unselected patients with chronic gonococcus vulvovaginitis, carefully catheterized specimens of urine showed a comparative absence of bacteria. The majority of the organisms encountered were either gram-positive cocci or diphtheroid bacilli. In most instances these were in such small numbers that they could easily be accidental contaminations from the urethra.

The second portion of the urine, representing the bladder flora, was shown to be as free from infecting organisms as was demonstrated in a previous series of normals. The occurrence of vulvovaginitis did not increase the tendency to contamination of the bladder.

Gonococcus cystitis may follow previous infections of the bladder by *Bacillus coli* in patients with vulvovaginitis.

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4. Colombini: Quoted by Young, H. H.: Johns Hopkins Hospital Rep., 1900, **9**, 677.

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AMERICAN MEDICAL ASSOCIATION
FIVE HUNDRED AND THIRTY-FIVE NORTH DEARBORN STREET
CHICAGO

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THE OCCURRENCE OF CITRIC ACID IN URINE

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With the intention of studying the fate of citric acid introduced in the organism by various routes and in various diseases, we tested the applicability of the method of Kunz to the quantitative determination of citric acid in urine and other body fluids. Briefly, the procedure of Kunz (1) is as follows: Evaporated wine is brought to a volume of 100 cc. under addition of 2 cc. dilute sulphuric acid and 20 cc. bromine water. To 50 cc. of the clear filtrate 10 cc. 1:1 H_2SO_4 and 3 cc. 37.5 per cent KBr solution are added. The flask is kept five minutes in a water bath of 50°. After removing from the bath 25 cc. of a 5 per cent $KMnO_4$ solution is run in from a pipette under vigorous shaking, not permitting the temperature during oxidation to rise above 55°. Finally, the remaining undissolved $Mn(OH)_4$ and the excess of bromine are removed by the addition of concentrated $FeSO_4$ acidified with H_2SO_4. Kunz then brings the precipitated pentabromacetone on a tared filter paper; washes well and dries in vacuo over H_2SO_4. He gives as the limit of his method 0.02 per cent citric acid ($C_6H_8O_7$ + 1 H_2O) that is 0.01 gram for the actual determination. We adhered to the procedure of Kunz inasmuch as the volume of the fluid before the addition of the strong H_2SO_4 and of the KBr was always 50 cc. We replaced the tared filter paper by Gooch crucibles, because filter paper blanks always lost from 2 to 4 mgm. weight and we dried the precipitate without the use of the vacuum, because it loses weight appreciably in vacuo. In a series of determinations the crucibles were left in the desiccator twenty-four hours and weighed. After another twenty-

odor of pentabromacetone. But when we tried to determine added citric acid in larger amounts, such as 100 to 300 cc. by evaporating the

TABLE 2

	CITRIC ACID + 1 H_2O MGM.		PERCENT RECOVERY
	Found	Added	
	49.0	50	98
	49.1		
	29.2	30	98
	29.3		
	18.9	20	95
	19.1		
A			
	8.3	10	86
	8.9		
	3.5	5	
	5.7?		
	1.6	2.5	60
	1.4		
	47.2	49.6	97
	48.9		
	28.6	30	96
	28.5		
	19.5	20	98
	19.8		
B			
	9.0	10	94
	9.8		
	3.8	5	81
	4.3		
	1.9	2.5	
	2.6		

urine and making up the filtrate from the residue to 50 cc., etc., we found that every normal urine, that of infants included, yielded a pre-

cipitate which could be identified as pentabromacetone, by its odor, its melting point of 73°, by the precipitation from its solution in acetone, alcohol and ether with water and by the fact that it could be distilled in a current of steam.

The question of the derivation of this pentabromacetone now arose. There are not many substances known to yield pentabromacetone easily. Meyer and Jacobson (2) mention besides citric and aceton dicarbonic acid, γ pyron and chelidonic acid. In all these cases, the pentabromacetone is finally derived from aceton dicarbonic acid.[1] Kunz reports satisfactory results with synthetic solutions containing tartaric, succinic, malic and lactic acid and glycerol, that is, none of these substances interfere with the reaction.

We tested among other substances, succinic, lactic, uric acid, l. β hydroxybutyric acid[2] separately and all with negative results. Neither did glycerol nor glucose nor a specimen of pyruvic acid (Kahlbaum) give any reaction. The pyruvic acid has been in the laboratory several years and such specimens are known to contain condensation products.

We had a specimen of campho glycuronic acid at our disposal prepared according to Schmiedeberg and Meyer (3) from the urine of rabbits injected with camphor dissolved in oil. The reaction was negative.

Tricarballylic, aconitic, ita, citra and mesaconic acid, more or less closely related to citric acid, did not yield any pentabromacetone. Peculiar results were obtained with two specimens of urine containing diacetic acid. One came from a diabetic patient, the other from a case of so-called cyclic vomiting. Of the diabetic urine 25 cc. boiled shortly with the addition of 1 cc. dilute H_2SO_4 did not give any reaction, while the unboiled urine gave a positive reaction.

The unboiled specimen of the urine from the second case gave a more pronounced reaction than the boiled. Diacetic acid, freshly prepared from its ethyl ester, was added to normal urine, and in some instances glucose was added also. The reaction seemed to be more pronounced when diacetic acid was added and in one series, where the controls did not give any reaction, the specimens with added diacetic acid gave a definite reaction with a little precipitate. In another series the controls gave a little precipitate and the specimens with the added diacetic acid seemed to show more turbidity and more precipitate, but there was only very little difference in the weight. A solution of

[1] Beilstein states it has been obtained from pyrotartaric acid with a melting point of 76°.

[2] We owe this acid to the kindness of Professor Shaffer.

diacetic acid shows a little peculiarity in the course of the reaction. On careful addition of the $KMnO_4$ there occurs a turbidity somewhat different from that seen in the usual reaction and on clearing the mixture with $FeSO_4$ none of the turbidity remains, in contrast to the behavior of citric acid solutions, where such a turbidity on addition of $KMnO_4$ is a sure indication of a positive reaction. Solutions of diacetic acid do not give a pentabromacetone reaction. Its presence in urine seems to have some influence on the reaction under certain hitherto unknown conditions.

The evidence is not absolutely conclusive that the pentabromacetone obtained from urine under the conditions of the experiment is derived from citric acid.

At the same time, until proof to the contrary is brought, we are fully justified in accepting citric acid as its source and therefore to claim citric acid as a constituent of normal human urine.

About this time Salant and Wise (4) published their method for the quantitative determination of citric acid based on the Denigès reaction. A number of urines from normal individuals and from patients were tested qualitatively in both ways and with exception of one gave both reactions. This urine from an atrophic infant gave a negative pentabromacetone reaction with over 200 cc. and it required the equivalent of 30 cc. urine to give a slight but definite Denigès reaction, when carried out according to Salant and Wise, the reaction being negative or doubtful with less. The positive Denigès reaction would be much stronger corroborative evidence for the presence of citric acid if it were not, like the pentabromacetone reaction, due to the production of aceton dicarbonic acid. Nevertheless, when we tested some of the substances mentioned above, substances giving a negative pentabromacetone reaction, we obtained positive Denigès reactions. These substances were pyruvic, or perhaps condensation products contained in our specimen, aconitic, ita- and citraconic acid.

It is of interest to note that neither tricarballylic nor mesaconic acid gave a reaction. The tricarballylic acid does not react at all on addition of the permanganate solution, the solution becoming and staying red after addition of the first drop. The itaconic acid was obtained as residue from a mixture of ita- and citraconic acid on distillation in a current of steam. Its melting point was 161° (uncorrected). The citraconic acid used for the tests was the oily part of the dry distillation of citric acid. The substance crystallized on evaporation, the crystals being of fatty appearance. Its melting point was not sharp, beginning

at 84°. The mesaconic acid was prepared by boiling a mixture of ita- and citraconic acid in 10 per cent NaOH for six hours. The melting point was about 200° (uncorrected.)[3] There are some differences in the course of the reaction when compared with the reaction of a citrate solution. It seems to require larger amounts particularly of aconitic acid to get a positive reaction. Furthermore, in the case of citrate solutions, the turbidity can be definitely seen, or when more concentrated, a precipitate is formed long before the color changes on addition of the $KMnO_4$. With the substances mentioned above, the precipitate becomes visible and seems to be formed on clearing with H_2O_2. Whether this precipitate is identical with that obtained from citric acid remains to be seen. It is doubtful whether the reactions just described detract from the value of the Salant and Wise method for the quantitative determination of citric acid in urine. But the fact that urine properly treated gives a positive pentabromacetone and a typical Denigès reaction, in the light of such occurrences, points very strongly to citric acid as the substance responsible for these reactions.

Taking into consideration the occurrence of citric acid in milk, it is not at all strange that it should also occur in urine. From the feeding experiments of Salant and Wise and from some of our observations on patients receiving large doses of sodium citrate per os, it is very improbable that the citric acid of the urine should come from citric acid contained in food. Dr. Rettinger, a pupil of Marchlewski, called our attention to a very interesting possibility, that is, the derivation of citric acid from hemopyrrol over an hematinic acid from which a partial anhydride of ethyl aconitic acid and ethyl tricarballylic acid have been obtained (5). This possibility would naturally include that of the derivation of citric acid in plants from chlorophyl.

Having established rather firmly the presence of citric acid in normal urine of infants, children and adults, it will be necessary to determine the amount excreted in a unit of time and its variations under various conditions. Some of this work has been started. Since 20 cc. of urine do not contain as a rule a sufficient quantity of citric acid to permit the use of the pentabromacetone method for its approximate quantitative determination, more urine can be taken. With normal urines, as much as 50 cc. filtered urine can be used under the arrangement of the experi-

[3] Citric acid heated with concentrated H_2SO_4 in a current of CO_2 gives CO, molecule per molecule. Tricarballylic, aconitic, ita—citra—and mesaconic acid all yield CO under such conditions, but not molecule per molecule, and the three last ones yield about the same amount, and more than the first two.

ments to which we adhered. To this 0.5 to 1.0 cc. H_2SO_4 may be added and bromine vapor dropped in. If no precipitate is formed, and thus far this never happened with normal urine, the pentabromacetone reaction can be carried out immediately. If the urine contains more than 10 mgm. citric acid rather good results may be obtained when compared with the results obtained by the method of Salant and Wise.

We will give a few examples later on. If the urine does not contain sufficient citric acid a larger amount may be evaporated with the addition of dilute H_2SO_4. The residue is taken up in water and the clear filtrate used for the determination. These solutions are sometimes very highly colored. To avoid this, we boiled the urine after the addition of dilute H_2SO_4 with animal charcoal. This procedure recommended itself furthermore for pathologic urines. The results obtained in some of the earlier experiments with the addition of citric acid to urine seemed to permit this process. Hereby a certain quantity of citric acid was added to urine, then some dilute H_2SO_4 and animal charcoal (Blutkohle Kahlbaum), the urine was boiled and filtered and of the filtrate a given amount was used. The residue on the filter was not washed. Of a sample containing 60 mgm. citric acid, 56.5 mgm. were determined and again another time 58.8 mgm. were added to urine, while 60.5 mgm. were found, here the control was negative, that is, it contained less than 5 mgm. citric acid.

In a series of experiments arranged as in table 2 the percentage recovery on addition of

50 mgm. citric acid was	99
30 mgm. citric acid was	95
20 mgm. citric acid was	88
10 mgm. citric acid was	77

Such results prompted us to employ animal charcoal in a number of experiments by preference. Later we found that sometimes the treatment with animal charcoal may entail considerable losses. Aqueous solutions of sodium citrate lose an appreciable amount of citric acid when treated with animal charcoal; for instance, from 30 mgm. citric acid, 21.7 mgm. and from 49.4 mgm. citric acid 41.1 mgm. were found, including the correction for the undetermined rest.

It seems that charcoal does not always adsorb citric acid from urine with the same avidity as from aqueous solutions.

The following table 3 gives the results of experiments as indicated. The figures represent milligrams citric acid found per 100 cc. urine. The

specimens of urine were mixtures obtained from several individuals. The first horizontal column gives the results obtained with 50 cc. filtered urine; the second, the results obtained with either 100 or 150 cc. urine after evaporation; in the third column, 250 to 350 cc. of urine were made strongly acid with sulphuric acid, animal charcoal was added and the urine boiled and filtered; of the filtrate 100 to 150 cc. were evap-

TABLE 3

Citric acid per 100 cc. in milligrams

(1) 50 cc. urine direct		20.5	21.2	29.1
(2) 100 to 150 cc. evaporated. No animal charcoal.	25.6	19.0	20.9	21.5
(3) 100 to 150 cc. filtrate from urine treated with animal charcoal	25.2	9.0?	21.0	25.5
(4) Filtrate from 100 to 150 cc. urine treated with charcoal residue washed		15.7	17.2	21.0
(5) Salant and Wise	31.3	22.2		29.5

orated, etc. In the fourth column 100 or 150 cc. of urine were heated with animal charcoal and filtered. The residue on the filter was taken up in boiling water; filtered and thoroughly washed with hot water. The washwater was added to the first filtrate, the total evaporated, etc. The fifth horizontal column contains the figures obtained by the method of Salant and Wise.

The results seem to indicate that the best results with the pentabromacetone method may be obtained using the urine without any further treatment, where that is possible. In how far the pentabromacetone method can be utilized for the quantitative determination of citric acid in urine has to be left to further work. While more time elapses to obtain the results, the method has the advantage over that of Salant and Wise in that it requires fewer and simpler manipulations.

In table 4 we give the results obtained with twenty-four hour specimens of urine from three normal adults (A., G. and R.). Urine was

TABLE 4

Citric acid in milligrams

	PENTABROMACETONE METHOD (ANIMAL CHARCOAL)		SALANT AND WISE	
	Per 100 cc.	Per twenty-four hours	Per 100 cc.	Per twenty-four hours
R.	47.0	475	50	505
G.	35.2	440	43	536
A.	21.8	440	27	548

taken for the determination by the method of Salant and Wise. The rest was boiled with animal charcoal and filtered. In the first two instances 50 cc. of the cooled filtrate served for the determination, in the last 300 cc. were evaporated.

It is seen that quite an appreciable amount of citric acid is excreted in the course of twenty-four hours, enough to play a modest rôle in the acid-base economy of the organism.

CONCLUSION

Normal human urine contains citric acid.

BIBLIOGRAPHY

(1) Kunz: Arch. Chem. Micr., 1914, vii, 285. Good abstract in Chem. Abst., 1915, ix, 687.
(2) Meyer and Jacobson: Lehrb. d. organ. Chem., 1913, i, 884.
(3) Schmiedeberg and Meyer: Zeitschr. f. physiol. Chem., 1879, iii, 422.
(4) Salant and Wise: Journ. Biol. Chem., 1916, xxviii, 27.
(5) See Nencki and Zaleski: Ber. chem. Gesellsch., 1901, xxxiv, 997.

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THE SCHNEYER METHOD FOR THE DETERMINATION OF LACTIC ACID IN URINE.

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(Received for publication, August 2, 1917.)

Meissner[1] in 1915 described a method for the determination of lactic acid in organ extracts, which Schneyer[2] has applied to the determination of lactic acid in urine. The method, as given by Schneyer, is as follows.

To 250 cc. of urine are added 2 cc. of cold saturated $CaCl_2$ and 15 to 20 drops of a 10 per cent ammonia solution. The mixture is heated and filtered. If it has been previously ascertained that diacetic acid and acetone are present, the filtrate is boiled for 15 minutes with mineral acid. The filtrate is then evaporated on a water bath to a syrup. The syrup is acidified with 5 cc. of H_3PO_4, about 13 per cent, and mixed with $CaSO_4$ to a dry crumbling powder. The powder is transferred quantitatively to an extraction thimble and extracted for 8 to 10 hours. The ether is evaporated from the extract and the residue is treated with concentrated H_2SO_4 in a current of carbon dioxide. The carbon monoxide evolved is collected in a nitrometer over 50 per cent KOH. The amount of lactic acid is calculated from the equation:

$$CH_3 - CHOH - COOH \rightarrow CH_3 - CHO + H_2O + CO$$

In our experiments, we followed the suggestion of Meissner, of testing the carbon monoxide for impurities by absorption in ammoniacal cuprous chloride solution.

Schneyer states that: (1) Hippuric and benzoic acids give no carbon monoxide on treatment with concentrated H_2SO_4.

(2) β-Hydroxybutyric and fatty acids develop carbon monoxide in such minute quantities that the error from this source may be neglected.

(3) Oxalic acid and diacetic acid evolve carbon monoxide in considerable amounts and are, therefore, to be eliminated—the oxalic acid by treatment with calcium chloride and ammonia, and the diacetic acid by boiling with mineral acid.

[1] Meissner, R., *Biochem. Z.*, 1915, lxviii, 175.

[2] Schneyer, J., *Biochem. Z.*, 1915, lxx, 294.

From the statements of Schneyer, the method seemed to fulfil its purpose better than any of the other indirect methods.

The values obtained frequently exceeded those obtained by other methods, so that one thought of the possible presence of substances, other than lactic acid, which were capable of yielding carbon monoxide. Schneyer reported no experiments, but promised the publication of results on normal and pathological urines. We had no opportunity of obtaining the later literature from abroad, and it is possible that Schneyer may in the meantime have recognized the limitations of the method.

Search was started for substances occurring in urine which could survive treatment with calcium chloride and ammonia, and which, when extracted with ether, would yield carbon monoxide.

TABLE I.

Date.	Patient.	Age.	Diagnosis.	Urine in 24 hrs.	Sp. gr.	Lactic acid.	
						Total excreted, Schneyer.	Corrected for hippuric acid.
		yrs.		*cc.*		*gm.*	*gm.*
Oct. 17, 1916.....	P.	6½	Endocarditis.	350	1020	0.1942	0.1630
" 31, 1916.....	B1.	8½	"	515	1010	0 5613	0.4945
June 7, 1917.....				815	1018	0.2575	0.1076
May 29, 1917.....	B2.	8	"	450	1020	0 1543	0.0651

Contrary to the statement of Schneyer, hippuric acid was found to give CO, due to the dissociated glycocoll which alone gives CO. The reaction does not proceed molecularly, but 0.1 gm. of hippuric acid was found to give consistently 0.0057 gm. of carbon monoxide. The amount of hippuric acid in the ether extracts of the urine was determined in a few cases by the method of Folin and Flanders.[3] From the amounts of hippuric acid found, the carbon monoxide from this source was calculated as lactic acid, and the original values for lactic acid were corrected. In Table I will be seen the values for lactic acid before and after this correction has been made.

It is evident that hippuric acid cannot be neglected offhand.

[3] Folin, O., and Flanders, F., *J. Biol. Chem.*, 1912, xi, 257.

Succinic acid liberates carbon monoxide easily when treated with sulfuric acid. When added to urine which was treated according to the Schneyer method, a decided increase in the amount of carbon monoxide was found. Succinic acid is said to occur in normal urines, and it has been found in pathological exudates (Hoppe-Seyler).[4] It is not at all unlikely, therefore, that some of the carbon monoxide may be derived from this source at times.

β-Hydroxybutyric acid, which occurs in normal urine of children[5] yielded a negligible amount of carbon monoxide in agreement with Schneyer's statement. Oxalic acid also yielded no carbon monoxide when added to urine which was treated according to Schneyer's method.

The values for lactic acid in normal urines which are given by indirect methods vary from 6 to 8 mg. per 100 cc. of urine. Ryffel,[6] who has written a very good review of the work done on the occurrence of lactic acid in urine under normal and pathological conditions, gives 6 mg. per 100 cc. of urine (sp. gr. 1020) as a normal value. Von Fürth[7] gives 8 mg. per 100 cc. as a normal average, using the bisulfite-iodine titration after clearing the urine with phosphotungstic acid and oxidizing with permanganate. Ishihara[8] also finds that a value of about 8 mg. per 100 cc. is normal. In non-pathological cases, the Schneyer method gave values very near the values obtained by these indirect methods. Table II shows the values obtained under different conditions by the Schneyer method.

Comparative determinations were made using the method devised by Ryffel,[9] a brief outline of which follows. 40 cc. of urine to which a known amount of lactic acid has been added are placed in a 500 cc. flask, and to this are added 45 cc. of concentrated sulfuric acid, rapidly through a dropping funnel, while the flask is shaken and cooled. The flask is connected to a steam

[4] Hoppe-Seyler, F., *Z. physiol. Chem.*, 1894, xix, 476.

[5] Veeder, B. S., and Johnston, M. R., *Am. J. Dis. Child.*, 1916, xi, 291. We are indebted for this acid to Professor Shaffer.

[6] Ryffel, J. H., *Quart. J. Med.*, 1909–10, iii, 221, 413.

[7] Von Fürth, O., *Wien. klin. Woch.*, 1914, 877.

[8] Ishihara, H., *Biochem. Z.*, 1913, l, 468.

[9] Ryffel, *J. Physiol.*, 1909–10, xxxix, p. v.

TABLE II.

Date.	Patient.	Age.	Urine in 24 hrs.	Sp. gr.	Lactic acid.		Remarks.*
					Total excreted, Schneyer.	Per 100 cc. of urine.	
		yrs.	cc.		gm.	gm.	
Mar. 22.	F1.	10	950	1015	0.0798	0.0084	Orthopedic operation on foot.
" 30–31....			1,135	1017	0.08502	0.0075	
	W.	11	560	1020	0.0554	0.0075	Operation after poliomyelitis.
Feb. 21.....	F2.	6	485	1012	0.03607	0.0062	Pneumonia.
" 29 ...			575	1010	0.1104	0.0192	
	M.	4	110	1020	0.0360	0.0330	Pneumonia, trace of acetone.
Mar. 2.....	S1.	9	520	1027	0.0925	0.0178	Pneumonia, urine very acid with some albumin and acetone.
" 5....			500	1027	0.1150	0.0230	Pneumonia, urine very acid with some albumin and acetone.
	S2.	8	372	1015	0.0457	0.0123	Pneumonia.
	A.	10	540	1014	0.0313	0.0058	Chorea.
Nov. 1.1916..	B1.	$8\frac{1}{2}$	735	1015	0.2947	0.0401	Endocarditis.
June 14, 1917..			410	1028	0.1683	0.0411	"

* Unless noted, no abnormalities in the urine were found.

generator and a condenser. A gentle current of steam is led into the flask, which is heated to 153–157° for 1 hour, or until 150 cc. of fluid have distilled. The distillate is made alkaline and redistilled. The second distillate is collected in a 100 cc. volumetric flask and compared colorimetrically with a standard prepared by the addition of Schiff's reagent (rosaniline hydrochloride decolorized with sulfurous acid) to a known solution of formaldehyde. I found it necessary to run a control experiment with a known solution of lactic acid to standardize the color produced by the Schiff's reagent with the formaldehyde each time the standard was made. This method shares, for very accurate work, the objections raised by Dehn[10] against all colori-

[10] Dehn, W. M., *J. Am. Chem. Soc.*, 1917, xxxix, 1392.

metric methods. But the criticism of Ishihara[8] with reference to the loss of acetaldehyde in the distillation was not borne out by experiments in which I used much smaller amounts of lactic acid.

Table III shows that it is possible to determine added lactic acid with the Ryffel method as well as with the Schneyer method.

TABLE III.

Date.	Patient.	Age.	Lactic acid.				Remarks.
			Per 100 cc. of urine.	Per 100 cc. of urine and lactic acid.	Found, added.	Added.	
Ryffel method.							
		yrs.	*gm.*	*gm.*	*gm.*	*gm.*	
Jan. 7, 1917..	B3.	13	0.0080	0.0200	0.0120	0.0119	Recovered chorea.
Nov. 14, 1916 .	B1.	8½	0.0095	0.0562	0.0467	0.0470	Determination made on ether extract of urine.
Jan. 8, 1917..			0.0100	0.0225	0.0125	0.0119	Endocarditis.
Schneyer method.							
Jan. 7, 1917..	B3.	13	0.0179	0.0298	0.0119	0.0119	Recovered chorea.
Nov. 14, 1916..	B1.	8½	0.0337	0.0568	0.0231	0.0230	Endocarditis.
Jan. 8, 1917..			0.0305	0.0440	0.0135	0.0119	"

After the correction had been made for hippuric acid as previously described, the values for lactic acid as determined by the Schneyer method were higher than those obtained with the Ryffel method, as in the following examples.

Date.	Patient.	Age.	Urine in 24 hrs.	Sp. gr.	Total lactic acid found in 24 hrs. by Schneyer's method.	After correction for hippuric acid. Ryffel and Schneyer.	Remarks.
1917		*yrs.*	*cc.*		*gm.*	*gm.*	
June 7.....	B1.	8½	815	1018	0.2575	R. 0.0509 S. 0.1076	Endocarditis.
May 29.....	B2.	8	420	1020	0.1543	R. 0.0180 S. 0.0651	"

THE NONPROTEIN NITROGEN OF THE BLOOD IN ATROPHIC INFANTS*

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The purpose of our work was the determination of the nonprotein nitrogen of the blood of infants, particularly of infants suffering from disorders of nutrition of the severer types. The blood was taken in quantities of 3 to 5 c.c. from the longitudinal sinus, following the directions of Helmholz.[1] The poor condition of most of our atrophic infants was the reason for taking as small quantities of blood as possible. In most of the cases the total nonprotein nitrogen and the urea nitrogen were determined, the former according to the method of Folin and Denis,[2] the latter by the Van Slyke and Cullen[3] modification of Marshall's[4] method. The portion of the alcoholic filtrate of the blood which is used for the determination of the urea nitrogen must be taken before the addition of zinc chlorid, because this substance interferes with the urease action. Creatin and creatinin were determined in a few of our normal and pathologic cases according to the method of Folin[5] as modified by Myers and Fine.[6] We found 1.1 to 1.6 mg. creatinin nitrogen and 3.0 to 7.6 mg. creatin nitrogen per 100 c.c. of blood, these figures agreeing well with those found in the table of Veeder and Johnston,[7] but subject to the criticism of Hunter and Campbell,[8] as our picric acid solution was old and not protected from light. In a few cases the amino-acid nitrogen was determined according to Van Slyke.[9] In four cases of our normal series and in five of the atrophic infants we found 3.5 to 7 mg. nitrogen per 100 c.c. of blood, figures agreeing with those obtained by Pettibone and Schlutz.[10]

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*From the Otho S. A. Sprague Institute Laboratory of the Children's Memorial Hospital.

1. Helmholz, H. F.: Am. Jour. Dis. Child., 1915, **10,** 194.
2. Folin and Denis: Jour. Biol. Chem., 1912, **11,** 527.
3. Van Slyke and Cullen: Jour. Am. Med. Assn., 1914, **62,** 1558.
4. Marshall: Jour. Biol. Chem., 1914, **17,** 351.
5. Folin: Jour. Biol. Chem., 1914, **17,** 475.
6. Myers and Fine: Chem. Composit. of the Blood, etc.
7. Veeder and Johnston: Am. Jour. Dis. Child., 1916, **12,** 136.
8. Hunter and Campbell: Jour. Biol. Chem., 1916, **28,** 335.
9. Van Slyke: Jour. Biol. Chem., 1915, **13,** 407.
10. Pettibone and Schlutz: Jour. Am. Med. Assn., 1916, **67,** 262.

Table 1 gives the results of total nonprotein and urea nitrogen determinations in mg. per 100 c.c. of blood. The sixteen infants, so-called feeding cases, were all doing well. The determinations were made before feedings, the infants being fed on mixtures of cow's milk at four-hour intervals. In most cases duplicate determinations were made, at least of the total nonprotein nitrogen, the duplicates agreeing rather well in most instances (cf. table). In Cases 6, 13 and 15 the difference exceeds 2 mg. It was not always possible to make duplicates of the urea nitrogen determinations, but where made, the agreement was quite good.

TABLE 1.—Convalescent Feeding Cases

No.	Name	Date	Age, Mos.	Wt., Lbs.	Mg. Total Nonprotein Nitrogen per 100 C.c. Blood		Mg. Urea N per 100 C.c. Blood	Remarks
1	Nel. O.	12/28/15	1	5.2	22.0	....	7.4	Discharged improved, 1/18/16
2	Tom. J.	3/ 3/16	2	7.0	23.6	23.6	11.5	
3	Florence Z.	2/18/16	3	9.10	26.2	26.0	....	Before a meal
	Florence Z.	.....	...	.....	27.9	27.5	....	After a meal
4	Tom. J.	4/16/16	3	8.6	23.0	23.0	12.1	Discharged; doing well
5	Kline F.	2/26/16	3	5.4	22.0	21.3	13.0	Premature; doing well
6	John K.	4/15/16	3	6.4	25.7	23.0	11.0	Discharged; doing well
7	Vernon G.	6/ 3/16	4½	7.13	24.8	24.5	13.5	Improved; doing well
8	James B.	12/21/15	5	8.6	23.0	22.4	....	Discharged; doing well
9	Elmer D.	3/18/16	5	11.3	25.9	25.2	13.1	Doing well
10	Bridget L.	12/18/15	6	10.2	23.4	21.8	14.4	Discharged; doing well
11	Magdalene G.	3/14/16	6	6.4	27.0	26.0	12.6	Discharged; doing well
12	Leon T.	4/20/16	6	10.8	24.3	....	....	Discharged; going well
	Leon T.	6/ 5/16	8	11	21.6	20.2	10.2	Discharged; doing well
13	Jos. N.	4/14/16	8	11.13	26.8	24.6	13.6	Discharged; doing well
14	Amelia P.	6/22/16	8	10.9	24.0	....	12.1	Discharged; doing well
15	Helen S.	6/13/16	10	10.10	24.0	21.4	8.8	Discharged; doing well
16	Malvina G.	2/24/16	16	12.8	20.0	18.2	11.0	Before a meal
	Malvina G.	.....	...	.....	27.3	26.3	12.0	After a meal

There is no uniformity of opinion in regard to the normal limits of the total nonprotein nitrogen of the blood in adults. Folin and Denis give 22 to 26 mg. per 100 c.c. of blood as the normal. Folin, Denis and Seymour,[11] Tileston and Comfort,[12] Frothingham and Smillie,[13] Rown-

11. Folin, Denis and Seymour: Arch. Int. Med., 1914, **13,** 224.

12. Tileston and Comfort: Am. Jour. Dis. Child., 1915, **10,** 278; ibid., Arch. Int. Med., 1914, **14,** 620.

13. Frothingham and Smillie: Arch. Int. Med., 1914, **14,** 541.

tree, Marshall and Baetjer,[14] and others accept as normal values those given by Folin and Denis,[2] or values varying between 20 and 30 mg. Others, as, for instance, McLean and Selling,[15] say the normal varies from 23 to 36 mg. Of greater interest to us here are the determinations in younger individuals. Schlutz and Pettibone[16] found in 9 infants from ½ hour to 10 days of age, 23 to 40 mg.; Veeder and Johnston, working on normal children from 2 to 14 years of age, get 22 to 33 mg. (5 exceeding 30 mg.); Leopold and Bernhard[17] get 19 to 40 mg. in 50 patients free from renal involvement, but suffering from various other diseases; the ages varied from 2 to 13 years; in 21 cases the total nonprotein nitrogen exceeded 30 mg. All these investigators used the method of Folin and Denis. Greenwald[18] objects to the use of alcohol in the precipitation of the blood, because the precipitate carries with it amino-acids and does not precipitate some lipoid nitrogen. In his determinations on 6 normal individuals, by the method of Folin and Denis and by his modification, he invariably finds higher values for the total nonprotein nitrogen by his method. In two instances his values exceed 30 mg. (31.0 and 31.7 mg.). Gettler and Baker[19] use hydrochloric acid and mercuric chlorid for the precipitation of the proteins. In their 30 patients, ranging from 17 to 60 years of age, they get from 27 to 52 mg. per 100 c.c. of blood, according to their table. Bang,[20] using his own method, not given in his paper, in 42 individuals got from 19 to 39 mg. The age of the individuals varied from new-born to 82 years. Although he gives 25 mg. as the average value, he got values greater than 30 mg. in 9 cases.

As Gettler and Baker[19] remark, the values for normal individuals given in the literature disagree more or less, and this may be due to the different methods used by the various workers, to the nature of the food or the time after a meal that the blood is taken. But quite aside from these factors is another very important one, namely, the latitude of the term "normal" individual. On the basis of our results we have been naturally very much inclined to accept as normal for the total nonprotein nitrogen of infants' blood, 22 to 30 mg. when determined by the Folin and Denis method. While our babies were doing well on nontherapeutic foods, they were convalescent from disorders of nutrition, and were, with few exceptions, decidedly underweight. *Sensu strictiori,* they are not normal, nor are many of the so-called normal

14. Rowntree, Marshall, Baetjer: Arch. Int. Med., 1915, **15,** 543.
15. McLean and Selling: Jour. Biol. Chem., 1914, **19,** 31.
16. Schlutz and Pettibone: Am. Jour. Dis. Child., 1915, **10,** 206.
17. Leopold and Bernhard: Am. Jour. Dis. Child., 1916, **11,** 432.
18. Greenwald: Jour. Biol. Chem., 1915, **21,** 61.
19. Gettler and Baker: Jour. Biol. Chem., 1916, **25,** 211.
20. Bang: Biochem. Ztschr., 1915, **72,** 104 and 119.

cases of other investigators, not even some of those of Gettler and Baker. It is not at all impossible that a somewhat more guarded selection of individuals may aid in doing away with the greater discrepancies.

In two of our cases (3 and 6) the determinations of the nonprotein nitrogen were repeated after feeding, Case 3 perhaps showing a slight, Case 6 a definite, increase similar to the cases of Tileston and Comfort,[12] who found an increase after a heavy meal of meat. Bang's[20] work on animals and man shows similar results.

Table 2 contains the results we obtained in atrophic infants arranged according to the amount of nonprotein nitrogen found. All these cases belong to a severe type of decomposition. With the exception of Cases 2 and 9, the urine did not show any of the ordinary pathologic changes; the urine of these two children contained pus. Of the 26 cases, 15 had more than 30 mg. nonprotein nitrogen per 100 c.c. of blood, and 11 had less than 30 mg. Of the 15 patients with more than 30 mg., 12 died and the other 3 did poorly when they left the hospital. Of the 11 cases of atrophy with less than 30 mg. nonprotein nitrogen, 3 patients died, 6 did fairly well or well, and 2 poorly. In our series of observations it is certainly a striking feature that of the 15 patients who died, 12 had a nonprotein nitrogen over 30 mg. and of these, 9 over 35 mg., while 3 fatal issues fell to the share of the group with less than 30 mg. nonprotein nitrogen. The occurrence of a relatively high nonprotein nitrogen in our cases may be the result of the interplay of a variety of factors, such as retention or overproduction in relation to the water economy of the organism. For instance, Bang,[20] in his animal experiments, has shown that starvation leads to a very marked increase in the nonprotein nitrogen, and particularly of the urea nitrogen, provided no water is given. Veeder and Johnston report one case of starvation with a rather scant increase of the nonprotein nitrogen (31 to 35 mg.). Bang mentions a loss of water by the blood in cases of starvation. We have not sufficient data to enter into a detailed discussion of the possible explanations of the relatively high nonprotein nitrogen in our cases. Only in a few cases did we determine the number of red cells and hemoglobin in order to detect a possible marked concentration of the blood. Such determinations were made on Cases 4, 7, 17, 23 and 24 — the first two having a relatively high nonprotein nitrogen, the last two a relatively low nonprotein nitrogen. In no case was the red count above 4,000,000 and the hemoglobin was always low. Aside from these determinations, the similarity of the cases with high or low nonprotein nitrogen does not speak in favor of a concentration of the blood as a factor in the production of the high nonprotein nitrogen, although the final decision must be left to a special investigation of this point. The possible influence of substances summarized in the total nonprotein nitrogen on the course of the disease will have to be considered in the future.

TABLE 2.—Atrophic Infants

No.	Name	Date, 1916	Age, mos.	Wt., Lbs.	Wt. Curve	Lect. T.	Mg. Total Nonprotein Nitrogen per 100 C.c. Blood		Mg. Urea N per 100 C.c. Blood	Remarks
1	Harold Ne.	3/17	2	5.8	S*	96.5	58.4	58.0	34.3	Died 4/6/16
2	Louise K.	4/16	9	10.2	L	99.0	52.4	52.0	29.5	Pus in urine; died 4/23
3	Regina M.	5/15	3	6.3	S	97.0	51.6	48.8	37.0	Died 6/17
4	Mazie M.	1/15	1	4.3	L	98.0	43.0	....	20.2	Died 2/7
5	Rosie C.	5/29	5	7.14	G	97.0	38.6	37.0	23.0	Died 7/17
6	Tom Mc.	4/12	3	6.7	L	96.0	38.0	37.3	21.8	Died 5/23
7	Esther W.	3/10	2½	6.3	G	97.0	37.4	36.0	16.0	Died 3/27
8	Louise H.	4/ 8	10	6.13	S	96.0	37.0	37.0	21.5	Died 4/16
9	John P.	4/17	4	7.4	S	100.0	36.0	....	15.5	
	John P.	5/10	5	7.2	S	98.0	31.7	....	15.0	Pus in urine; improved, 6/17
10	Mollie F.	2/25	7	6.10	L	97.0	35.3	34.7	17.6	
	Mollie F.	7/18	12	6.12	L	98.0	38.8	....	20.3	Died 7/18
11	Rose P.	7/17	5	8.2	G	97.0	34.8	33.0	20.0	Doing poorly 7/30
12	Beverley G.	2/12	4	5.3	S	96.0	34.0	29.4	12.7	Died 3/1
13	Richard S.	3/15	2	5.12	S	97.0	33.3	32.8	10.0	
	Richard S.	5/18	4	5.12	S	98.0	29.7	....	10.4	Doing poorly 7/5
14	Fred W.	1/31	3	6.10	L	97.0	32.4	....	15.9	Died 2/19
15	Walker W.	1/24	8	7.12	L	97.0	30.7	....	....	Died 1/30
16	Myrtle D.	6/ 2	7	6.12	L	97.0	29.7	29.0	10.0	Died 6/19
17	John S.	5/13	8	7.12	S	100.0	29.0	24.9	8.0	
	John S.	5/27	8½	8.4	G	98.0	26.7	....	10.8	Doing fairly well 6/8
18	Harold Na.	4/19	8	7.9	L	96.0	26.9	26.4	12.3	Died 4/23
19	Ray S.	12/30	6	7.12	L	97.0	25.2	24.8	12.0	Died 1/8
20	Alice T.	6/28	8	9.0	S	98.0	24.8	22.6	10.8	Doing fairly well 7/14
21	Helen M.	7/12	2½	5.6	S	98.0	24.3	22.8	13.6	Doing poorly 7/30
22	Harry B.	4/26	6	6.10	G	97.0	23.1	....	8.9	Doing well 7/15 (9 pounds)
23	Robert S.	3/10	2½	6.0	S	98.0	23.0	20.6	6.1	
	Robert S.	4/29	4	7.8	S	98.0	19.5	19.3	5.6	
	Robert S.	5/ 3	...	7.8	S	100.0	21.2	....	7.7	Doing well 6/20
24	Helen S.	5/ 1	5	5.4	S	99.0	22.6	....	7.6	Doing poorly
25	Nellie O.	12/28	1	5.2	S	96.0	22.0	....	7.4	
25	Nellie O.	4/23	5	5.3	S	96.0	25.0	....	7.7	Doing fairly well 7/1
26	Louise K.	7/11	3	5	G	97.0	21.5	21.3	7.3	Doing well 7/28

* S = weight curve stationary when blood was taken; L = losing weight; G = gaining weight.

With reference to the relationship of the urea nitrogen to the total nonprotein nitrogen, Table 3 gives a survey of our series of cases. A is calculated from Table 1, leaving the figures obtained after feeding out of consideration. B is calculated from those cases of Table 2, wherein the nonprotein nitrogen exceeds 30 mg., and B2 from the remainder of Table 2, using in Cases 23 and 25 the averages for the calculation.

TABLE 3.—Summary of Authors' Cases

	Nonprotein N	Urea N	Nonprotein N Minus Urea N
A (16 cases)	24	12	12
B (15 cases)	39	20	19
B2 (11 cases)	25	10	15

It is a rather precarious undertaking to utilize averages from a relatively small number of cases which show quite extensive variations. We will, therefore, not dilate on the possible significance of these figures any further than to point out that the burden of the increase of the nonprotein nitrogen does not seem to be borne preponderantly by the urea nitrogen.

Another circumstance may be of significance: Where a relatively low nonprotein nitrogen was associated with an urea nitrogen markedly below 50 per cent. of the nonprotein nitrogen, the infants were, on the whole, getting on rather well (of Cases 17, 22, 23, 24, 25 and 26, only Case 24 did poorly). If such an occurrence should prove to be a concomitant feature of certain phases of the reparation from disorders of nutrition, it could easily explain the low urea nitrogen of Cases 1 and 15 of Table 1.

TABLE 4.—Alimentary Intoxication

No.	Name	Date	Age, Mos.	Wt., Lbs.	Mg. Total Nonprotein Nitrogen per 100 C.c. Blood		Mg. Urea N per 100 C.c. Blood	Remarks
1	John O.	2/12/16	13	21.0	67.6	....	31.7	Died 2/13; Temp. 106
2	Eugene A.	3/17/16	5	14.10	60.0	57.5	48.3	Died 3/18; edema
3	E. Smith	2/20/16	7	15.4	25.6	....	11.0	Feeding case; discharged well, 3/2/16
	E. Smith	4/ 5/16	8½	11.0	53.0	....	37.2	Died 4/7
4	John J.	4/21/16	4	9.9	29.4	....	16.1	Died 4/24
5	Leon C.	7/13/16	12½	17.2	28.0	27.2	18.4	Died 7/19
6	Loren H.	2/27/16	9	13.14	26.7	26.4	17.3	Died 3/1

Table 4 contains the results obtained in some cases of alimentary intoxication.

The urines of all the patients contained albumin and casts, so that in the cases with high nonprotein nitrogen (Cases 2 and 3) a renal insufficiency may play its rôle. Case 3 is of interest. When the infant was in the hospital the first time, suffering with a mild disturbance of nutrition, its nonprotein nitrogen and urea nitrogen were about normal. Six weeks after its discharge it returned in a state of acute alimentary intoxication, when its values were high. In all but one of our cases of intoxication the urea nitrogen was decidedly more than 50 per cent. of the total. The blood of the first three children was obtained within twenty-four to forty-eight hours before death; in the other cases it was obtained at least three days before death.[21]

CONCLUSIONS

1. The blood of infants recovering from the milder intestinal disorders contains between 20 and 30 mg. of nonprotein nitrogen per 100 c.c. as determined by the method of Folin and Denis, agreeing with the values obtained by several authors for normal individuals. The urea nitrogen forms about 50 per cent. of the nonprotein nitrogen.

2. In atrophic infants the nonprotein nitrogen frequently exceeds 30 mg. per 100 c.c. of blood, and these findings seem to be associated rather frequently with a bad prognosis.

3. A low urea nitrogen associated with a low nonprotein nitrogen in atrophic infants seems to be a favorable sign.

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21. Dumitrescu and Popescu (Zentralbl. f. Biochem. Biophys., 1915, **18,** 184. Abstr., Chem. Abstr., 1916, **10,** 931) described a very marked increase of the total nonprotein nitrogen and urea in the blood shortly before death in patients suffering for some time of disease other than renal. The original paper is not at our disposal and the abstract does not contain any further data of use to us in this connection. Among some determinations in various conditions we have one made on an infant of 9½ months, weighing 16 pounds, which died of a capillary bronchitis on the day the determination was made. The nonprotein nitrogen was 24 mg., the urea nitrogen 13 mg., that is, there was no increase.

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AMERICAN MEDICAL ASSOCIATION
FIVE HUNDRED AND THIRTY-FIVE NORTH DEARBORN STREET
CHICAGO

[Contribution from the Otho S. A. Sprague Memorial Institute and the Pathological Laboratory of the University of Chicago.]

THE MECHANISM OF THE NINHYDRIN REACTION.

A CONTRIBUTION TO THE THEORY OF COLOR OF SALTS OF ALLOXANTINE-LIKE COMPOUNDS.[1]

By J. M. Retinger.

Received November 27, 1916.

Ninhydrin is the name given by Abderhalden to triketohydrindenehydrate, which because of its peculiar color reaction with amino acids and amines, is used by him as indicator in his dialysis method for detection

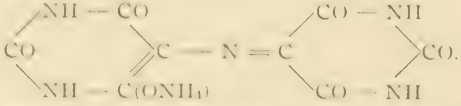
[1] The experimental work upon which this article is based was completed at the University of Leipzig in 1913 under the direction of Prof. A. Hantzsch and appears among the publications of that institution. Out of courtesy to Prof. Hantzsch with whom it is impossible to communicate at present, the author does not feel justified in quoting in detail from the protocols.

After submission of this paper for publication Prof. J. Stieglitz called my attention to a publication by G. N. Lewis in the Proceedings of the National Academy of Sciences, 1916, p. 586–92, in which a very similar idea for color production in special cases

of the activity of specific ferments in the animal organism under pathologic conditions.

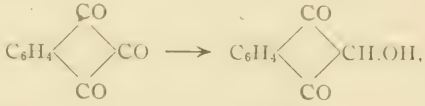
In all the recent theoretical papers dealing with the mechanism of the ninhydrin reaction a parallel is drawn between the substance causing the blue coloration and murexide. The substance having the purple color is said to be diketohydrindylidene diketo hydrindamine, although it has been neither isolated nor identified as such. The only evidence supporting that assumption is the similar way of preparing that compound and the qualitative identity of the absorption in the visible part of the spectrum.[1]

Alloxan with ammonia gives murexide

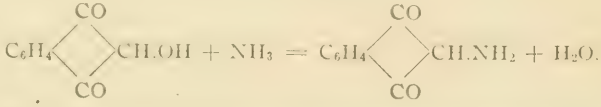


The reaction between triketohydrindene and amino acids is similarly explained:

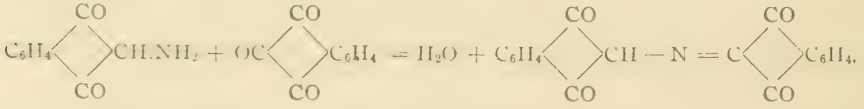
"The nitrogen atom is supplied by the decomposition of the amino acid into the corresponding glyoxal and ammonia, the former causing a reduction of the triketone to 1,3-diketohydrindole



which condenses with loss of water with the ammonia



A second molecule of triketohydrindene hydrate then condensed with the 1,3-diketohydrindamine



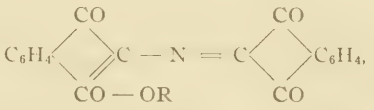
to give diketohydrindylidene-diketohydrindamine."

is advocated. In discussion of the problem of the color of triphenylmethyl, G. N. Lewis makes, on page 589, a statement that "there seem, therefore, to be every reason to believe that triphenylmethyl is colored not because it assumes the quinoid form, but because it possesses an odd electron which, as in every other known substance of this class except NO, has a sufficiently low natural frequency to absorb visible light." In other words the colorless hexaphenylethane dissociates into two triphenylmethyls because of overloading of the affinities of the middle carbons with heavy groups as shown by Schlenck (*Ann.*, **272**, 1 (1910)) and the presence of a free valence or odd electron determines the color. That, of course, applies also to alloxantine and hydrindantine and explains the reaction in an identical way from the electronic point of view.

[1] Harding and Warneford, *J. Biol. Chem.*, **25**, 319–336 (1916); Harding and McLean, *Ibid.*, **25**, 337–350 (1916).

The authors themselves see a difficulty in explaining the reaction with organic amines because the question of supplying the necessary unsubstituted nitrogen for the bridge would necessitate a splitting off of the amino group, which is known to be a matter of great difficulty if not an entire impossibility under these conditions.

Likewise the similarity of absorption in the visible spectrum, when the absorption as stated, is not selective, *i. e.*, composed of several bands all over the spectrum, but uniform going from red to green, cannot be a criterion of identity. Any two substances dissolving with the same color and shade would have the same absorption in the visible spectrum, provided the absorption is not selective, because then the visible color would be the result of mixing of many single colors. In this case the absorption would be represented by one single broad absorption band. But even here, a number of Abderhalden reactions, which will be the subject of another paper, have given evidence that not only the shade but also the color would not always be identical. Its tint varies from blue to purple, a fact which could hardly be explained by assuming the murexide-like formula for the coloring matter



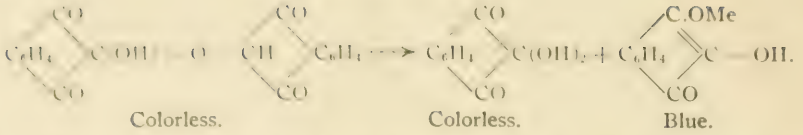
because in that case the change of color would be caused by a colorless cation R while we know that esterification or salt formation does not as a rule deepen or lighten the absorption. The difference in the constitution of the amine can of course have no influence on the bridge-nitrogen as all its three valencies are necessary for the bridge connection and a pentavalent nitrogen would behave in general in a very different way.

The diketohydrindylidene-diketohydrindamine explanation of the ninhydrin reaction not being sufficient for elucidation of all facts connected with it, an attempt may be made to explain it in a quite different way, basing the theory on observations made by S. Ruhemann[1] on hydrindamine and on experimental work by the author on the colored salts of alloxantine and hydrindantine.[2]

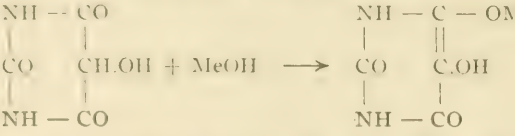
In 1911 S. Ruhemann made the observation that hydrindantine gives a red coloration with potassium carbonate and a blue one with free potassium hydroxide. But this coloration was so unstable that all attempts to isolate the colored substance failed. He declared the red production be a true salt of hydrindantine, but the blue substance to be a salt of the dioxindone. According to his theory by splitting from hydrindantine, triketohydrindene and dioxindone are formed, and the latter is enolized by the alkali and gives the chromo-salt

[1] S. Ruhemann, *J. Chem. Soc.*, **99**, 792, 1306 (1911).

[2] J. M. Retinger, *Dissertation*, Leipzig (1913).



Ruhemann gives no proofs to support his views but nevertheless takes it as base for a conclusion that alloxantine is decomposed in the same way by barium hydroxide, that it is split into alloxan and dialuric acid and that the latter is enolized and gives the purple chromo-salts.[1]

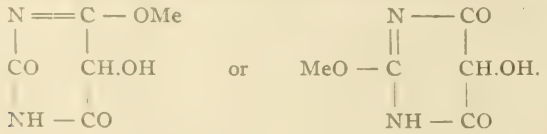


The explanation is untenable according to my experimental work on the purple salts of alloxantine previously cited.[2] In addition to the Ba salts, also the K, Na, and Rb salts of the simple alloxantine, its two dimethyl derivatives, and of malic acid, *i. e.*, tetramethyl-alloxantine, were prepared, though not all in a state of purity suitable for analysis. These salts regenerate, on acidification under exclusion of oxidation, alloxantine and not dialuric acid. In the same way potassium dialurate when treated with an excess of barium hydroxide does not yield the violet supposed to be basic dialurates.

On acidifying the blue alkaline solution of hydrindantine no hydrindantine is obtained, a fact which is explainable by the great lability of the colored substance. After disappearance of the coloration, dioxindone and *o*-carboxyl-mandelic acid remain in the liquid just as from alloxantine and aqueous potassium hydroxide dialuric and alloxanic acids are formed. Even in the alloxantine series the purple salts remain undestroyed only, when they are insoluble, as the Ba salts in the alkaline mother liquor, or as the alkali salts in alcohol. As soon as they go into solution (as the alkaline salts in water) they are destroyed.

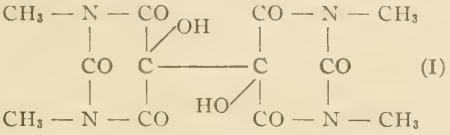
Of the colored salts the following were prepared in analyzable state of purity: The Na and Ba salts of the simple alloxantine and of its two dimethyl homologs; all those salts have four metallic equivalents for every molecule of alloxantine and are, of course, formed by rearrangement of two NH-CO groups into the enolic form N = COH.

[1] He gives the colorless dialurate the very unbelievable formula



[2] *Loc. cit.*

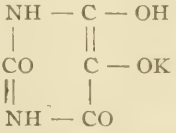
Amalic acid treated with $Ba(OH)_2$ under all precautions gives also blue but very perishable salts, although as a tetramethyl-alloxantine,



(I)

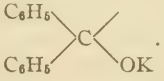
it contains only two substituable hydrogens—those in the hydroxyls.[1]

From this fact the conclusion can be drawn, that the color of those salts is not due to the enolization of the NH-CO groups but to primary salt formation on the free hydroxyls and especially on the second one because monometallic salts are colorless.[2] The enolic hydroxyl of the formula by Piloty cannot be considered as giving the coloration to the salts because the potassium salt of dialuric acid



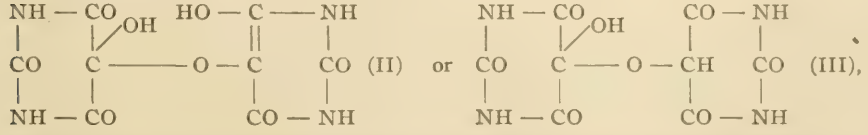
remains colorless in alkaline liquid although it contains an identical configuration with the other part of alloxantine.

On the basis of newer researches a satisfactory, though radical explanation, can be given to the formation of color in these salts. Schlenk and Zeickel[3] ascribe to the blue potassium salt of benzophenone the formula with a trivalent carbon or as we may say with a free valency



In salts of alloxantine we would assume the affinity of the two middle carbons becomes so loosened by the vicinity of four negative oxygen-holding groups that their connection is severed very easily, even by substitution of both of their hydroxyls by metals.

[1] I ascribe to alloxantinethe old Formula I with carbon-binding between the two parts and not that of Piloty (*Ann.*, **333**, 62 (1904)) II or Slimmer and Stieglitz III (THIS JOURNAL, **31**, 661 (1904)),

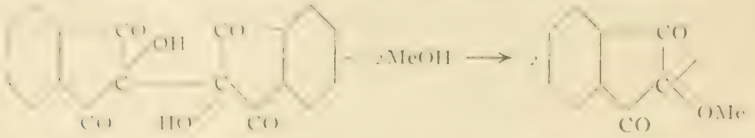


because in that case we would have a quinhydrone-like compound which ought to be deeper colored than its "quinoid" component, while according to the work of Bilz (*Ber.*, **45**, 3659 (1912)) the anhydrous alloxan is yellow while alloxantine is colorless.

[2] J. M. Retinger, *Dissertation*, Leipzig (1913).

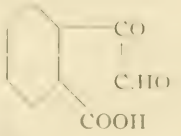
[3] Schlenk and Zeickel, *Ber.*, **44**, 1182 (1911); Beckmann and Paul, *Ann.*, **266**, 1 (1891); K. H. Meyer, *Ibid.*, **377**, 62 (1904).

The same explanation applies to the blue salts of hydrindantine

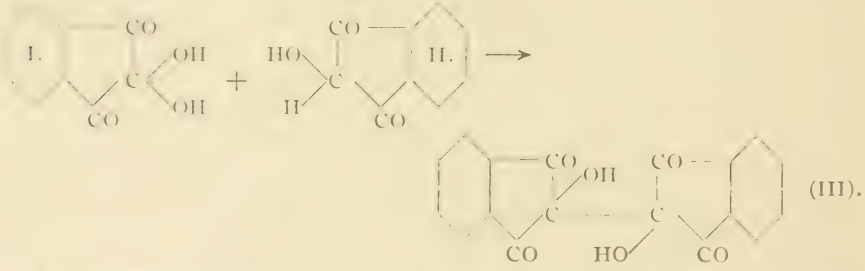


because in the salt formation with hydrindantine also only the grouping CO — C(OH) — CO takes action while the benzole rest which is connected with it has no significance at all, just as the urea rest in alloxantine is of no importance for the chromo-salt formation.

Ruhemann has shown that triketohydrindene hydrate partly hydrolyzes into *o*-carboxyl-phenylglyoxal



which as a rather strong reducing agent, on account of its two carbonyl groups, will reduce one molecule of triketohydrindene to dioxindone (II). This cannot exist in a free state in the presence of triketohydrindene (I), and consequently will combine with the excess of it to give hydrindantine (III).



Hydrindantine in its turn when reacted upon by an amino acid will give the salts in which on account of overloading of three valencies of the bridge carbons with heavy groups the remaining valencies will not be sufficiently strong to hold the two halves of the molecule together. The two halves will split apart and remain free, thus forming two independent molecules or radicals. The great instability of the colored substances in all the cases mentioned is a great support to the theory. This free valency would have to be responsible for the color of the combination and there can hardly be any objection to the use of the idea of a trivalent carbon after the magnificent work of Gomberg in that field.

It was mentioned already that in different ninhydrin reactions the color of the liquid ranges from pink to deep blue-violet and it was pointed out that the simple esterification or salt formation could not account for the

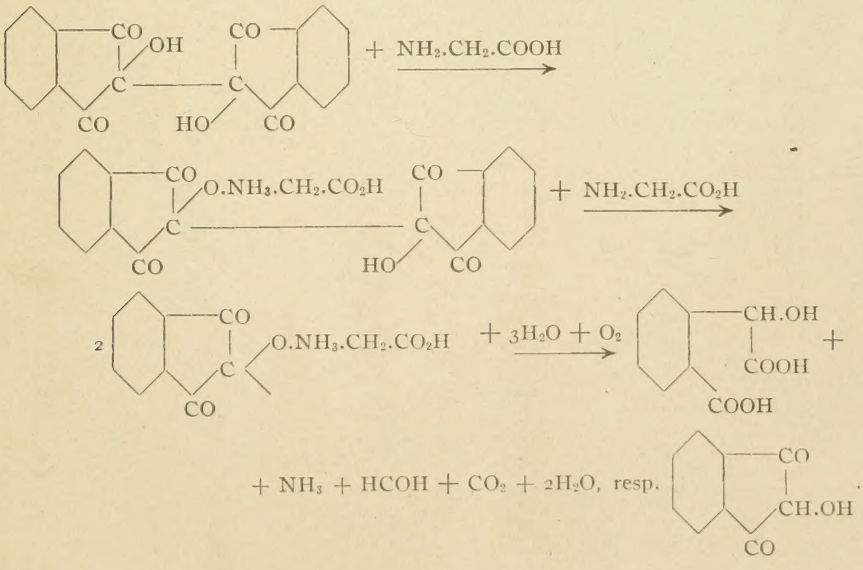
difference in color since the cations in that case are colorless and could not produce that effect. In our explanation there is also a salt formation but the conditions vary. The groupings connected with the trivalent carbon are of different sizes and consequently take up different amounts from the sum of its valencies; the remaining free affinity varies in size or, we may say, has a different potential, and produces a different optical effect in every instance.

The colored liquids resulting from a positive ninhydrin reaction, if left exposed to the air for several days, fade and finally become colorless. If we accept the diketohydrindylidene-diketohydrindamine explanation for the ninhydrin reaction there is no reason for this fading, as this compound is stable. The trivalent carbon formula explains this fact just as well as the instability of the blue salts of alloxantine.

The chromo-salt of hydrindantine with the amino acid undergoes hydrolysis and oxidation giving *o*-carboxyl-mandelic acid, ammonia, carbon dioxide and an aldehyde which in turn is partly used up for reducing the other part of the molecule to dioxindone. After acidifying the solution, we have *o*-carboxyl-mandelic acid and dioxindone, just as in the case of alloxantine alloxanic and dialuric acids.

Summary.

The whole ninhydrin reaction would then proceed as follows: The triketohydrindene hydrate hydrolyzes during boiling giving *o*-carboxyl-glyoxal which reduces part of the triketohydrindene to dioxindone, which in turn combines with another molecule of triketohydrindene to give hydrindantine. The amino acid or amine derived from enzyme action



gives first, as shown in the alloxantine series on alkaline salts, a monobasic salt which is colorless; further boiling produces a dibasic neutralization, and the molecule splits into equal parts with trivalent carbon—a free valency—as cause of the absorption in the visible spectrum. Exposure to air in water solution decomposes the split molecules further, giving *o*-carboxyl-mandelic acid, ammonia, carbon dioxide, water and an aldehyde, resp., dioxindone through reduction.